THE AMERICAN JOURNAL OF PHYSIOLOGY

VOLUME LXVIII

BALTIMORE, MD.
1924

Med.
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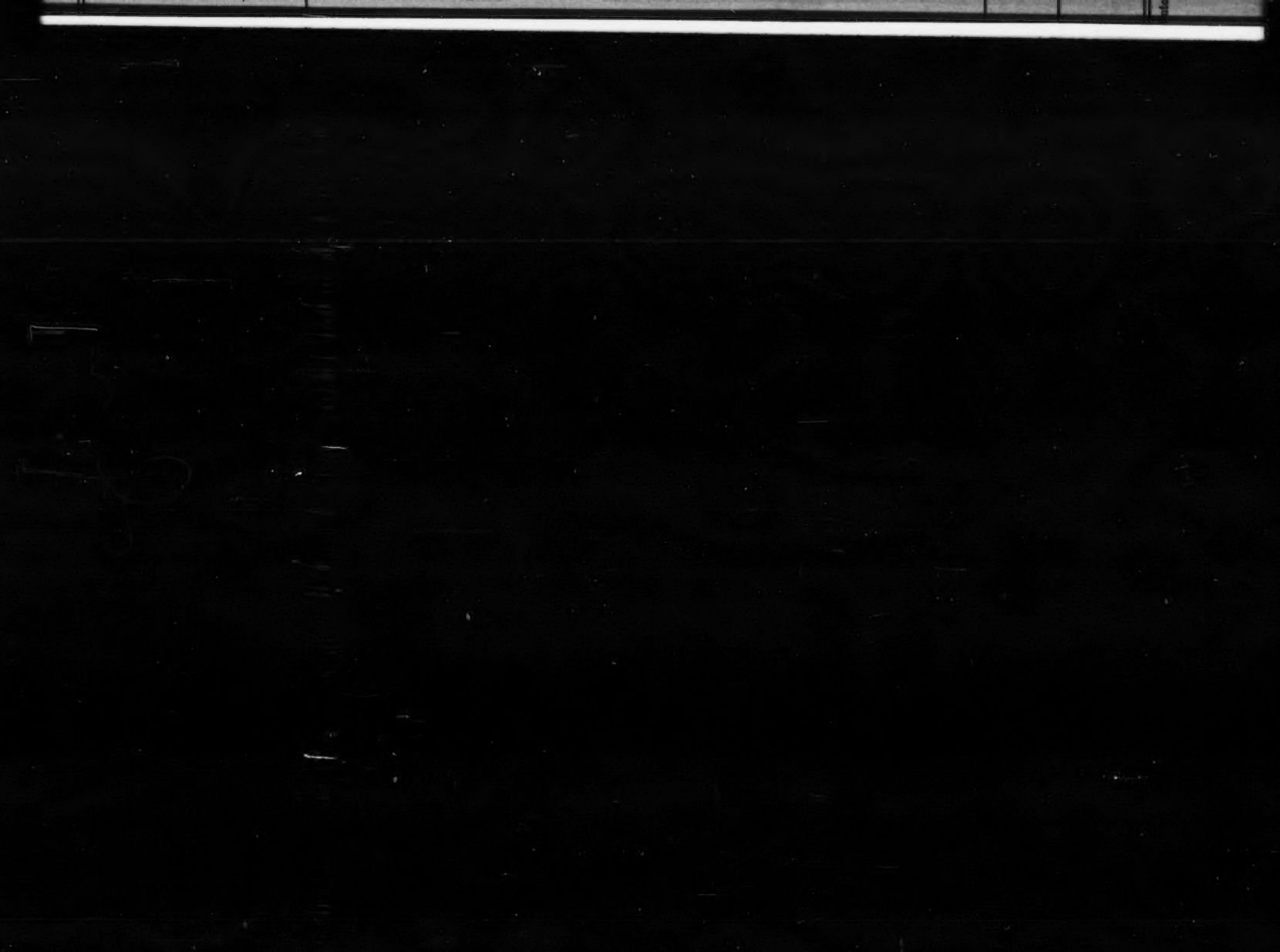

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THE AMERICAN JOURNAL OF PHYSIOLOGY

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THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. LXVIII—No. 1

Issued March 1, 1924

BALTIMORE, U. S. A.

1924

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918.

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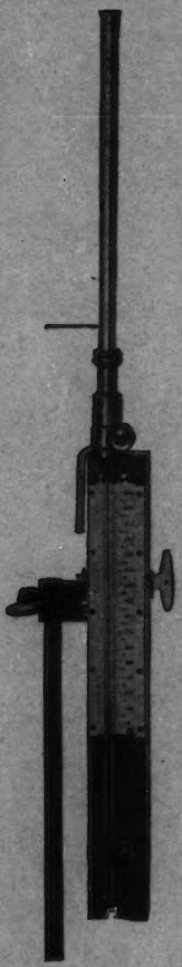

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VOL. 68 MARCH 1, 1924 No. 1

STUDIES OF THE THYROID APPARATUS. XIX

The Effects of the Loss of the Thyroid and Parathyroid Glands at 75 Days of Age on the Gross Growth of the Albino Rat

FREDERICK S. HAMMETT

From The Wistar Institute of Anatomy and Biology, Philadelphia

Received for publication November 27, 1923

This paper continues the report of the rôle of the thyroid apparatus in growth from the point of view of the age and sex factors. The data on body length, body weight and tail length of rats thyro-parathyroidectomized or parathyroidectomized when 75 days of age are its subject matter.

The age of 75 days was chosen as a point of departure because the albino rat is approaching the completion of the pubertal adjustment at this time.

The details as to the distribution, care, diet and environment of the animals, and the general plan and scope of the study were given in the paper dealing with the effects of thyroid and parathyroid deficiency initiated at 100 days (1). The same criteria used in that communication apply to this. In comparing the two series, the one which is the main subject of this report will be called the 75 day old series, that of the earlier paper the 100 day old series.

A total of 208 rats was used. Owing to incomplete removal of the thyroid or the parathyroids, or to death before the 150 day age had been reached, the final number available was 88. Their distribution is given in table 1.

It frequently happened that at the end of the period of observation there remained of a given litter only the control and a thypar or parathy rat. I have, therefore, used the mean values of the litter controls of the test animals of the three groups (operated controls, thypars and parathys) as the basis of comparison. By this the variability due to random sampling is eliminated. There is no need to advance any argument for the desirability of using litter controls in studies of this type.

The growth of the male rats. Before analyzing the course of growth in body weight during the 75 day interval of observation (75 to 150 days), the differences in body length, body weight and tail length of the three groups will be discussed.

Table 2 contains the data for such comparison.

From table 2 it is seen that:

1. There was a general identity in body length, body weight and tail length of the test groups with their controls at 75 days of age. This permits direct comparison of the terminal values.

2. At 150 days of age the rats of the operated control group did not differ from their litter controls in mean body length, body weight or tail length. The operative procedure as such has no harmful effect upon gross growth. Therefore no further attention will be paid to this group at this time.

3. At 150 days the thypars were shorter, lighter and had shorter tails than did their litter controls. Thyroid loss retards gross growth. The thypars of this series were practically the same size as the thypars of the 100 day old series.

TABLE 1

The distribution of the rats of the 75 day old series

	CONTROLS	OPERATED CONTROLS	THYPARS	PARATHYS	TOTALS
Males	14	6	12	13	45
Females	16	5	12	10	43
Totals	30	11	24	23	88

4. At 150 days the parathys were shorter, lighter and had shorter tails than did their litter controls. Parathyroid deficiency retards gross growth. The parathys of this series were smaller than those of the 100 day old series. A lack of parathyroid function from 75 days of age until 150 days of age is evidently more harmful to gross growth than is a similar deficiency existing from 100 to 150 days of age. An analysis of this with respect to growth in body weight will be given presently.

5. At 150 days the parathys were lighter and tended to be shorter than the thypars. A parathyroid deficiency initiated at 75 days of age is more inhibitory of growth than is a thyroid deficiency similarly established.

6. In all groups at all times the variability in body weight was greater than that in body length or tail length. Moreover the variability in body weight was increased by thyroid and parathyroid loss, while the variability in body length and tail length was not. This supports the belief expressed before (1), that growth in cell volume is more sensitive to disturbances of metabolic origin than is growth in cell number.

TABLE 2

The statistical data of the body length, body weight and tail length of the male rats of the various groups

	BODY LENGTH		BODY WEIGHT		TAIL LENGTH	
Age, days	75	150	75	150	75	150
Controls for operated controls, 6 rats						
	mm.	*mm.*	*grams*	*grams*	*mm.*	*mm.*
Mean	164.5	204.5	124.3	228.9	144.0	172.8
S.D.	11.1	9.5	19.3	31.8	11.4	8.8
P.E.M.	3.1	2.6	5.3	8.7	3.2	2.4
C.V.	5.7	4.6	15.6	13.9	7.9	5.1
Operated controls, 6 rats						
Mean	166.8	204.3	133.2	227.9	146.8	170.8
S.D.	12.9	7.3	21.2	27.5	11.0	5.6
P.E.M.	3.6	2.0	5.8	7.6	3.0	1.6
C.V.	7.8	3.6	15.9	12.1	7.5	3.3
Controls for thypars, 10 rats						
Mean	167.5	206.4	133.8	246.0	144.6	173.9
S.D.	10.3	11.4	17.9	35.5	9.2	8.1
P.E.M.	2.2	2.4	3.8	7.6	2.0	1.7
C.V.	6.2	5.5	13.3	14.4	6.4	4.7
Thypars, 12 rats						
Mean	169.8	186.7	137.2	183.1	148.3	159.9
S.D.	13.3	12.0	27.3	52.0	9.7	8.8
P.E.M.	2.6	2.3	5.3	10.1	1.9	1.7
C.V.	7.8	6.5	19.9	28.4	6.6	5.5
Controls for parathys, 9 rats						
Mean	171.1	208.4	139.9	257.0	151.6	178.1
S.D.	13.1	9.5	30.0	34.6	11.9	8.4
P.E.M.	2.9	2.1	6.8	7.8	2.7	1.9
C.V.	7.6	4.6	21.5	13.4	7.8	4.7
Parathys, 13 rats						
Mean	167.7	182.2	132.0	147.2	147.2	159.1
S.D.	13.9	12.4	31.6	40.4	11.1	11.0
P.E.M.	2.6	2.3	5.9	7.6	2.1	2.1
C.V.	8.3	6.8	24.0	27.5	7.6	6.9

TABLE 2—*Concluded*

The males of the 100 day old series as at 150 days of age

	BODY LENGTH			BODY WEIGHT			TAIL LENGTH		
	Controls	Thypars	Parathys	Controls	Thypars	Parathys	Controls	Thypars	Parathys
Mean	202.5	186.5	189.9	229.8	180.0	192.8	172.2	159.3	163.9
S.D.	5.3	10.5	9.1	26.3	36.2	33.5	7.0	11.6	10.4
P.E.M.	1.1	2.0	1.6	5.3	7.0	6.0	1.4	2.3	1.9
C.V.	2.6	5.6	5.0	11.4	20.1	17.4	4.1	7.3	6.4

S.D., Standard deviation.
P.E.M., Probable error of mean.
C.V., Coefficient of variability.
Contl., Control.
Thyp., Thypar.
Para, Parathy.

A convenient method for the determination of the relative effects of experimental procedures on the growth of the various parts of the organism has been outlined in a previous paper (1). In table 3 are to be found the values for the absolute G.C. (growth capacity) of the various groups and in table 4 the G.C. of the test groups in terms of that of their controls. Similar data from the 100 day old series are given for comparison. The method gives the percentage increment of growth for the stated period of observation and the value is called the absolute growth capacity (G.C.).

TABLE 3

The absolute G.C. of the body in length and weight, and of the tail in length for the 75 day period of observation. Males

	CONTROLS FOR THYPARS	THYPARS	CONTROLS FOR PARATHYS	PARATHYS
Body length	23.2	10.0	21.8	8.6
Body weight	83.9	33.5	83.7	11.5
Tail length	20.3	7.8	17.5	8.1

From table 4 it is evident that:

1. The combined loss of the thyroid and parathyroid glands at 75 days of age retards growth in body weight to a much less degree than does the loss of the parathyroids alone. The retardation in body and tail length is of the same order of magnitude in the two groups. The body weight relation is the opposite from that obtaining in the 100 day old series. It suggests that age is a factor.

2. The tendency for growth in body weight (cell size) to be retarded to a greater degree than growth in body or tail length (cell number) by thyroid and parathyroid deficiency is shown here as in the 100 day old series. It is support for the idea that growth by increase in cell number is less susceptible to disturbance than is growth in increase in cell size.

3. The retardation of tail length growth is of the same order of magnitude in both groups of both series as is that of body length. This might have been expected from the nature of the structures involved.

Is age a factor in the sensitivity of the animals to the growth retardation following thyroid and parathyroid deficiency?

An inspection of table 4 shows that in the rats deprived of the thyroid apparatus when 75 days of age the growth in body length and body weight was less retarded than it was in rats similarly treated when 100 days old. The younger animals are more resistant to the growth inhibiting effects of thyroid deficiency than are the older ones.

This quantitative difference in response may be due to the greater strength of the G.C. in the younger animals, to the longer period allowed for recovery, or to an age difference in metabolic sensitivity. It was pointed out in the preceding paper of this series (2) that the possibility exists that in animals of the same age but differing in G.C., those with the stronger G.C. are more resistant to inhibition of growth than those with

TABLE 4

The G.C. in body length, body weight and tail length of the various groups of males in terms of that of their controls

	75 DAY OLD SERIES			100 DAY OLD SERIES		
	Controls	Thypars	Parathys	Controls	Thypars	Parathys
Body length	100.0	43.1	39.4	100.0	35.2	64.0
Body weight	100.0	39.9	13.7	100.0	31.0	57.1
Tail length	100.0	38.4	46.3	100.0	37.2	63.9

the weaker. An analysis of the growth in body weight with reference to age and weight as functions of the G.C. shows that the strength of the initial G.C. is also a factor in the age differences of response.

In the rats deprived of their parathyroid glands when 75 days of age the growth in body length, body weight and tail length, was much more retarded than was it in rats from which these structures were removed at 100 days. The younger males are less resistant to the toxemia of parathyroid deficiency than are the older ones.

Turning now to a detailed examination of the course of growth in body weight, there are given in table 5 the statistical data which serve as the basis for such a study. The mean values for the weekly measurements are plotted on charts 2 and 3 together with those of the 100 day old series for comparison. On chart 1 are plotted the values of the coefficient of variability on age as given in table 5.

From chart 1 it is evident that the variability in body weight tends to be increased by thyroid and parathyroid lack, and probably to a greater degree by the latter.

An important point shown by chart 2 is that the removal of the thyroid apparatus at 75 days of age does not produce the immediate cessation of growth that follows the same procedure in rats 100 days old.

Chart 3 brings out several points of interest. It shows that for the first 50 days after parathyroid removal the contour of the curves is approximately the same for both the 75 day old and 100 day old series. The preliminary loss of weight due to the acute toxemia of parathyroid deficiency occurs in both groups and is followed by recovery. The weight of

TABLE 5

Summary of the weights of the four groups of males at each period of measurement

DAY	75	82	89	96	103	110	117	124	131	138	145	150
						Controls for thypars						
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Mean	133.8	156.1	169.4	181.7	193.6	203.6	212.6	219.0	227.7	233.5	240.6	246.0
S.D.	17.9	22.9	23.9	22.5	26.1	27.0	27.6	29.1	32.2	33.1	34.3	35.5
P.E.M.	3.8	4.9	5.1	4.8	5.6	5.8	6.1	6.2	6.9	7.1	7.3	7.6
C.V.	13.3	14.7	14.1	12.4	13.5	13.3	13.4	13.3	14.1	14.2	14.3	14.4
						Thypars						
Mean	137.2	139.1	147.1	151.7	157.3	160.3	165.5	167.7	173.6	175.9	178.7	183.1
S.D.	27.3	35.2	33.7	31.5	36.3	39.6	42.4	43.3	47.0	45.8	45.5	52.0
P.E.M.	5.3	6.9	6.6	6.1	7.1	7.7	8.3	8.4	9.2	8.9	9.4	10.1
C.V.	19.9	25.3	22.9	20.8	22.6	23.9	25.3	24.9	26.7	26.0	27.1	28.4
						Controls for parathys						
Mean	139.9	159.5	175.9	188.9	202.4	213.8	225.7	231.9	240.3	246.8	254.7	257.0
S.D.	30.0	32.5	30.9	30.1	31.2	32.3	31.8	33.7	34.0	33.4	34.6	34.6
P.E.M.	6.8	7.3	6.9	6.8	7.0	7.3	7.1	7.6	7.7	7.5	7.8	7.8
C.V.	21.5	20.4	17.6	15.9	15.4	15.1	14.1	14.6	14.2	13.5	13.6	13.4
						Parathys						
Mean	132.0	122.1	125.6	133.4	139.7	144.8	148.4	150.7	149.7	148.4	148.2	147.2
S.D.	31.6	29.0	29.1	33.4	31.2	36.0	35.8	33.1	34.6	37.7	39.3	40.4
P.E.M.	5.9	5.4	5.4	6.3	5.8	6.7	6.7	6.2	6.5	7.1	7.4	7.6
C.V.	24.0	23.8	23.1	25.0	22.3	24.9	24.1	21.9	23.1	25.4	26.5	27.5

the rats deprived of these glands at 75 days of age begins to fall off from 124 days of age on, slowly it is true, but surely. This phenomenon did not occur in the group parathyroidectomized when 100 days old, but it is possible that if the period of observation had been extended a similar result would have been obtained. This decline in weight of the younger rats suggests a cumulative effect of the toxemia. Finally it should be noted that the weight of the parathys of the 75 day old series fell much further below that of their controls than did that of the parathys of the 100 day old series.

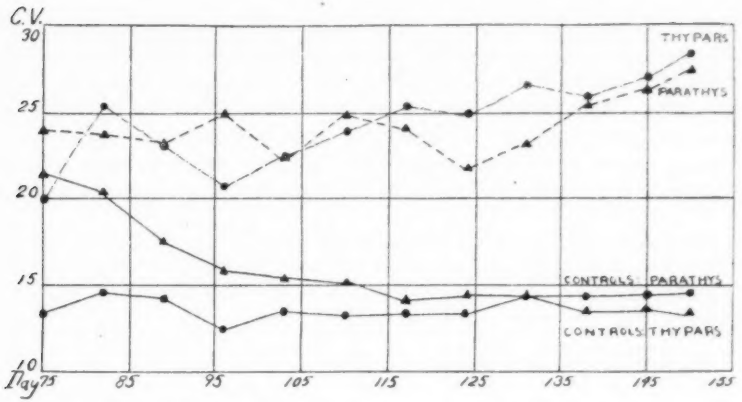

Chart 1. The coefficient of variability on age of the male thypars and parathys and their controls.

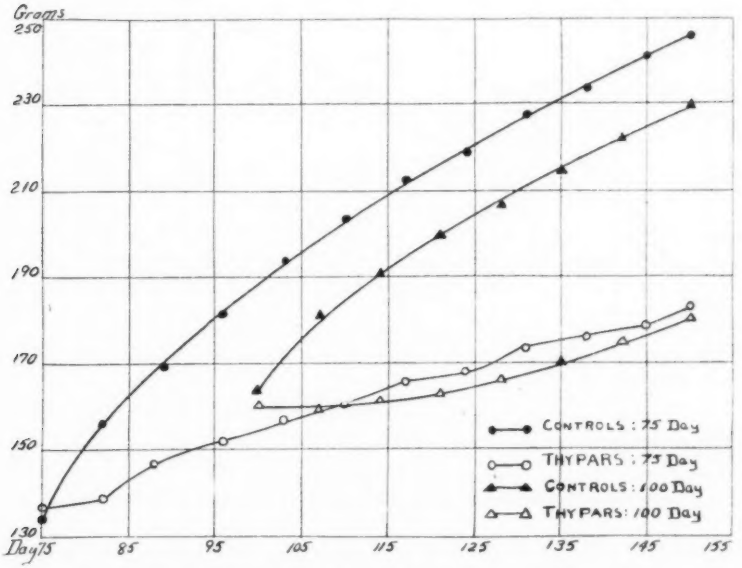

Chart 2. The weight on age of the male thypars and their controls

In my earlier discussion of the idea of growth capacity, attention was focused upon its change with age. It was shown that the G.C. decreases regularly with age. It is observed that since rats grow heavier as they grow older the G.C. also decreases with increasing body weight.

Now if all rats were the same weight at the same age it would be possible to consider the G.C. as a function of age or weight and the expression for the value of this function in the one would be the expression for its value in the other. But rats are not turned out with machine-like regularity as to size and growth ability. It is possible, even from the same parent stock, to obtain groups of animals which have distinctly different mean weights at the same age. This is shown by the curves for the two sets of controls

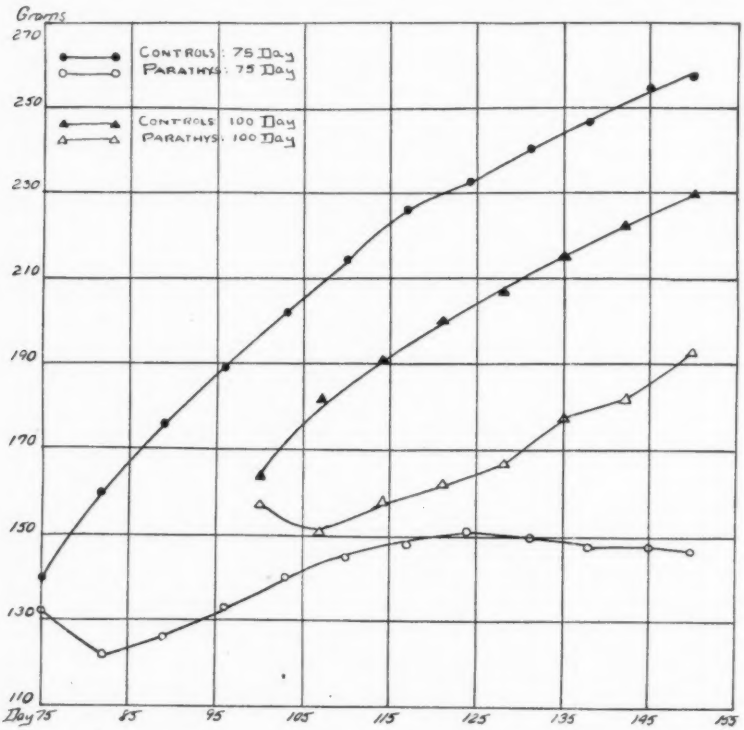

Chart 3. The weight on age of the male parathys and their controls

in chart 2. The rats of the 100 day old series were consistently lower in weight, age for age, than were those of the 75 day old series. Such being the case it seemed desirable to attempt an evaluation of the G.C. both as a function of age and as a function of body weight as an aid in the analysis of the results produced by the experimental procedures. The application of the results of the analysis in an interpretation of normal growth will be reserved for a later communication.

Inspection shows that the curves drawn by the growth of the control groups are of an exponential type. It was found that the observations fitted the type formula

$$(1) \qquad W = Ae^{\frac{K}{1+nt}}$$

when W = weight at a given time, t; A the calculated maturity weight, and K and n constants. In the evaluation of the equation three points on the growth curve are taken. The type formulae for the computation of n, K and A are as follows:

$$(2) \qquad M = \frac{\log_e \frac{W_3}{W_1}}{\log_e \frac{W_2}{W_1}}$$

$$(3) \qquad n = \frac{t_3 - t_1 - M\ (t_2 - t_1)}{Mt_3\ (t_2 - t_1) - t_2\ (t_3 - t_1)}$$

$$(4) \qquad K = \frac{\log_e \frac{W_2}{W_1}}{\frac{1}{1+nt_2} - \frac{1}{1+nt_1}}$$

$$(5) \qquad A = We^{-\frac{K}{1+nt}}$$

The equations for the control curves are given in table 6. Differentiation of 1 for G.C. (grams per 100 grams per day in this case) as a function of time (age) gives the type formula

$$F_t = -\frac{100\ Kn}{(1+nt)^2}$$

and for G.C. as a function of mass (body weight):

$$F_m = -\frac{100\ n}{K}\ [\log_e W - \log_e A]^2$$

By the use of these formulae and the equation in table 6 the normal expected values for the G.C. as related to age and as related to body weight of the rats of the test groups at each age and weight of record are computed. The values so obtained are those which the test rats should have exhibited had they grown as did their litter controls. The per cent difference between these and those actually obtaining in the test groups affords a quantitative basis for intergroup comparison. The data for the males are given in table 7.

TABLE 6

The equations for the growth curves of the control groups.

	75 DAY OLD SERIES		100 DAY OLD SERIES CONTROLS
	Controls for thypars	Controls for parathys	
Males	$W = 365.9e^{\frac{1.8842}{1-0.0383t}}$	$W = 371.5e^{\frac{1.5136}{1-0.034t}}$	$W = 315.6e^{\frac{0.6088}{1-0.0198t}}$
Females	$W = 256.2e^{\frac{4.9247}{1-0.0974t}}$	$W = 239.1e^{\frac{3.5097}{1-0.0733t}}$	$W = 218.6e^{\frac{0.3870}{1-0.0189t}}$

TABLE 7

The expected and the observed G.C. and their per cent difference. Males

AGE	75 DAY OLD SERIES						100 DAY OLD SERIES					
	Thypars			Parathys			Thypars			Parathys		
	Expected	Observed	O/E	Expected	Observed	O/E	Expected	Observed	O/E	Expected	Observed	O/E
G.C. as function of age												
75	2.06	0.20	9.7	2.14	−1.07	−50.0						
79	1.76	0.56	31.8	1.81	−0.26	−14.4						
86	1.37	0.60	43.8	1.39	0.70	50.4						
93	1.10	0.50	45.5	1.10	0.76	69.1						
100	0.90	0.37	41.1	0.89	0.59	66.3	1.36	−0.04	−2.9	1.36	−0.54	−39.7
107	0.75	0.39	52.0	0.74	0.43	58.1	1.03	0.13	12.6	1.03	0.71	68.9
114	0.64	0.30	46.9	0.62	0.27	43.5	0.82	0.17	20.7	0.82	0.36	43.9
121	0.55	0.37	67.3	0.53	0.04	7.5	0.66	0.23	34.8	0.66	0.40	60.6
128	0.47	0.31	66.0	0.46	−0.10	−21.7	0.54	0.43	79.6	0.54	0.91	168.5
135	0.41	0.21	51.2	0.40	−0.07	−17.5	0.46	0.39	84.8	0.46	0.33	71.7
142	0.37	0.39	105.4	0.35	−0.09	−25.7	0.39	0.34	87.2	0.39	0.77	197.4
Mean			51.0			15.1			45.2			81.6
G.C. as function of body weight												
	1.96	0.20	10.2	2.14	−1.07	−44.4						
	1.90	0.83	43.7	2.78	0.41	14.7						
	1.69	0.44	26.0	2.64	0.89	33.7						
	1.58	0.53	33.5	2.36	0.67	28.4						
	1.45	0.27	18.6	2.15	0.53	24.7	1.46	−0.04	−2.7	1.55	−0.54	−34.8
	1.38	0.46	33.3	1.99	0.36	18.1	1.47	0.13	8.8	1.73	0.71	41.0
	1.28	0.19	14.8	1.89	0.23	12.2	1.43	0.17	11.9	1.51	0.36	23.8
	1.24	0.50	40.3	1.83	−0.10	−5.5	1.38	0.23	16.7	1.40	0.40	28.6
	1.13	0.19	16.8	1.86	−0.13	−7.0	1.32	0.43	32.6	1.29	0.91	70.5
	1.09	0.23	21.1	1.89	−0.01	−0.5	1.20	0.39	32.5	1.05	0.33	31.4
	1.04	0.49	48.1	1.90	−0.14	−7.4	1.10	0.34	30.9	0.97	0.77	79.4
Mean			27.9			6.1			18.7			34.3

In order that the age comparison could be made on a more exact basis the observed mean weights of the test groups of the 75 day old series at the stated ages were adjusted by interpolation to fit the ages at which the measurements were made of the 100 day old series. Both the observed and the expected G.C. values represent grams per 100 grams per day.

The analysis of the body weight growth of the males. The removal of the thyroid apparatus and the parathyroids from male rats 75 and 100 days old reduces the G.C. both as related to body weight and as related to age.

In both groups of both series the average reduction was much greater in the G.C. as a function of body weight. Those processes of growth in which the time factor is predominant were less disturbed than those which are related to body mass.

It is fair to assume that the retardation of growth following thyroid and parathyroid removal is due to disturbances of metabolism. In the one case there is a lowering of the metabolic level, in the other, a toxic inhibition of the normal predominance of the anabolic processes associated with increase in body mass. It is clear that this reduction is exerted to a greater extent on the G.C. as related to body weight than on the G.C. as related to age. Therefore, it is probable that the former is more dependent upon assimilatory metabolism for its expression than the latter. Growth is obviously the expression of the sum total of a number of chemical reactions. It is clear that the G.C. as a function of body weight is intimately related to the mass of reacting substances, it is probable that the G.C. as a function of age (time) is an expression of the mean velocity of the chemical reactions. I will reserve further expansion of this analysis for a later discussion. The possibility should be noted that growth by increase in cell number may be a factor of greater importance in the expression of the G.C. as a function of age than in the expression of the G.C. as a function of body weight. Cognizance should be taken of the fact that the G.C. in its relation to age is also, in the normal animal, an expression of assimilatory metabolism.

The reduction in the G.C. as a function of body weight is less marked when the thyroid apparatus is removed at 100 days. On the other hand the cut in the G.C. as a function of age is of the same order of magnitude in both series. Therefore the greater G.C. of the thypar rats of the 75 day old series, as shown in table 4, is largely due to the greater resistance of their mass-G.C. function. Although the difference between the G.C.-age relation is in the same direction as in the G.C.-body weight relation and it is thus possible that relation plays some part in the series difference in total G.C., the conclusion is justified that age (as such) plays a minor rôle in the greater G.C. of the younger thypars. From this is derived the conclusion that the greater the assimilatory metabolism the greater the resistance to growth retardation following thyroid (thypar) deprivation.

The greater total G.C. of the rats thyro-parathyroidectomized at 75 days of age can hardly be attributed to the longer period of survival, because the initial effects on the G.C. (time) were much less marked in the thypars of the 75 day old series than in those of the 100 day old series, and their recovery toward the normal values failed to be as marked in the later intervals.

The male parathys of the 75 day old series differed from those of the 100 day old series in that at no time did the observed G.C., on either basis, reach the expected value; from the fact that negative values obtained in the later periods, and from the fact that the reduction in the G.C. was greater than that produced by thyroid (thypar) deficiency.

The lack of parathyroid secretion for the 75 day interval from 75 to 150 days of age caused a greater reduction in the G.C. as a function of both age and weight than did the lack for the 50 day interval in the rats operated when 100 days old. Was this an age, mass or cumulative response?

When the mean degree of retardation of the G.C. as a function of age of the two series is compared with that of the G.C. as a function of body weight, it is evident that the relation of the reduction of the latter to the former is practically the same in both series. Therefore since neither the G.C. as a function of age or the G.C. as a function of body weight is predominantly responsible for the age differences in growth inhibition due to the toxemia of parathyroid deficiency, it is concluded that the lesser growth of the younger rats is a consequence of a heightened sensitivity accompanying the particular stage of development occurring at the time the deficiency was initiated, in which the G.C. as a function of age and the G.C. as a function of body weight participate.

A cumulative effect of the toxemia is also a factor, but not the only factor, in the younger rats, as shown by the regression of the G.C. during the last three periods of observation, a phenomenon which was not exhibited by the animals parathyroidectomized at 100 days of age. That age is a factor is shown by the fact that the regression in growth immediately following the removal of the parathyroids was greater in the younger rats and that they failed to approach the normal rate of growth as closely as did the parathys of the 100 day old series during the succeeding periods of observation.

The G.C. both as a function of age and as a function of body weight was reduced to a greater degree in the parathys (75 day old series) than in the thypars. The degree of inhibition was greater in the G.C. as a function of body weight. This shows that the growth retardation in the parathys is due to a greater disturbance in the G.C.-mass relation than occurred in the thypars. That is to say, the interference with the metabolic processes concerned with growth as related to body weight, was absolutely and relatively more severe in the parathys than in the thypars.

The growth of the female rats. Table 8 contains the data for the discussion of the differences in body length, body weight and tail length of the various groups of females. Similar data from the females of the 100 day old series at 150 days of age are included for comparison. The material is comparable with that given in table 2 for the males.

TABLE 8

The statistical data of the body length, body weight and tail length of the female rats of the various groups

	BODY LENGTH		BODY WEIGHT		TAIL LENGTH	
Age	75	150	75	150	75	150
Controls for operated controls, 5 rats						
Mean	164.4	186.2	124.8	164.5	152.8	161.8
S.D.	8.9	9.1	15.9	22.1	7.2	3.7
P.E.M.	2.7	2.7	4.8	6.7	2.2	1.1
C.V.	5.4	4.9	12.8	13.4	4.7	2.3
Operated controls, 5 rats						
Mean	162.0	190.8	124.8	170.6	154.0	163.2
S.D.	9.0	8.2	15.6	12.2	7.0	7.2
P.E.M.	2.7	2.5	4.7	3.7	2.1	2.2
C.V.	5.6	4.3	12.5	7.2	4.5	4.4
Controls for thypars, 10 rats						
Mean	161.6	189.4	117.3	178.5	146.7	163.2
S.D.	8.3	4.4	18.8	15.8	10.7	6.3
P.E.M.	1.8	0.9	4.0	3.4	2.3	1.4
C.V.	5.1	2.3	16.0	8.8	7.3	3.9
Thypars, 12 rats						
Mean	159.4	170.9	112.6	123.0	147.4	152.4
S.D.	12.7	9.7	25.5	32.9	13.1	10.5
P.E.M.	2.5	1.9	5.0	6.4	2.5	2.0
C.V.	7.9	5.7	22.7	26.8	8.9	6.9
Controls for parathys, 8 rats						
Mean	158.5	185.5	109.5	168.5	145.0	162.1
S.D.	5.7	6.6	12.8	23.3	3.7	2.9
P.E.M.	1.4	1.6	3.1	5.5	0.9	0.7
C.V.	3.6	3.6	11.7	13.8	2.5	1.8
Parathys, 10 rats						
Mean	158.8	172.3	114.4	132.9	148.6	159.7
S.D.	7.0	9.6	13.0	25.5	6.6	7.2
P.E.M.	1.5	2.0	2.8	5.4	1.4	1.5
C.V.	4.4	5.6	11.4	19.2	4.4	4.5

TABLE 8—*Concluded.*

The females of the 100 day old series as at 150 days of age

	BODY LENGTH			BODY WEIGHT			TAIL LENGTH		
	Controls	Thypars	Parathys	Controls	Thypars	Parathys	Controls	Thypars	Parathys
Mean	190.2	176.0	177.5	177.0	130.8	144.1	163.5	156.6	157.3
S.D.	6.5	7.7	10.6	21.2	22.7	24.5	6.2	6.0	10.3
P.E.M.	1.3	1.4	1.8	4.3	4.2	4.4	1.3	1.1	1.8
C.V.	3.4	4.4	5.9	12.0	17.3	17.0	3.8	3.8	6.5

From the table it is seen that:

1. There was a general identity in body length, body weight and tail length of the test groups with their controls at 75 days of age. The size of the rats of the various groups at the beginning of the period of observation was sufficiently uniform to allow direct comparison at the terminal stage.

2. At 150 days of age the rats of the operated control group did not differ from their litter controls in mean body length, body weight or tail length. In the females, as in the males, the operative procedure as such has no harmful effect upon the gross growth. Therefore no further attention will be paid to this group in this paper.

3. At 150 days the thypars were shorter, lighter and had shorter tails than their litter controls. As in the males, so also in the females, thyroid loss retarded gross growth. The thypar females of this series were smaller than were those of the 100 day old series.

4. At 150 days of age the parathys were shorter and lighter than their litter controls. As in the males, so in the females, parathyroid deficiency retards gross growth. The parathys of this series tended to be smaller than those of the 100 day old series.

5. At 150 days of age the parathys tended to be somewhat larger than the thypars, a condition opposite to that observed in the males, but similar to that which obtained in the 100 day old series.

6. In all groups at all times the variability in body weight was greater than that in body length or tail length. The interpretation and the supportive evidence is the same as that recorded for the males.

In table 9 are to be found the absolute growth capacities of the parts measured, for the various groups, and in table 10 the values of the test groups in terms of that of their controls, together with similar data from the 100 day old series for comparison. The material is comparable with that given for the males in tables 3 and 4.

From table 9 it is seen that the absolute G.C. of the normal female rat in body length, body weight and tail length is less than that of the normal male during the growth period from 75 to 150 days of age. The same was found to be the case in the rats observed from 100 to 150 days.

Table 10 shows that:

1. The combined loss of the thyroid and parathyroid glands retards growth in body length, body weight and tail length to a greater degree than does the loss of the parathyroids alone. This finding is in agreement with that obtained from the 100 day old series. It is the opposite of that observed in the males. Moreover the lack of parathyroid secretion retards gross growth in the females to a lesser degree than it does in the males, an opposite reaction to that obtaining in the 100 day old series. There is therefore both a qualitative and a quantitative sex difference in response to a parathyroid deficiency initiated at 75 days of age.

TABLE 9

The absolute G.C. of the body in length and weight, and the tail in length for the 75 day period of observation. Females

	CONTROLS FOR THYPARS	THYPARS	CONTROLS FOR PARATHYS	PARATHYS
Body length	17.2	7.2	17.0	8.5
Body weight	52.2	9.2	53.9	16.2
Tail length	11.2	3.4	11.8	7.5

TABLE 10

The G.C. in body length, body weight and tail length of the various groups of females in terms of that of their controls

	75 DAY OLD SERIES			100 DAY OLD SERIES		
	Controls	Thypars	Parathys	Controls	Thypars	Parathys
Body length	100.0	41.9	50.0	100.0	24.5	47.2
Body weight	100.0	17.6	30.1	100.0	−28.2	20.7
Tail length	100.0	30.4	63.6	100.0	19.4	49.3

2. The tendency for growth in body weight to be more retarded than growth in body length or tail length is shown in the females of both groups and to a more marked degree than in the males. It is similarly interpretable

3. The retardation of tail length growth with respect to body length growth is more marked in the female than in the male thypars. The interpretation of this phenomenon as a forwarding of essential growth, and the basis therefor, was given in a previous paper (1). While the interpretation is strengthened by these results, a certain lack of consistency exhibited by the male thypars weakens the general conclusion, the substantiation of the validity of which must await further data.

4. Comparing the male and female thypars the former are seen to have been generally more resistant to retardation of growth than the latter.

This is particularly marked in the body weight growth. The differences in body length growth are negligible of themselves but the direction is significant in supporting the above conclusion. Here as in the 100 day old series the female rats are more sensitive to inhibition of growth by thyroid deprivation than are the males, and that of the three factors studied the body weight is the most sensitive and the body length the least.

Now as to the quantitative differences in response attributable to age factors.

1. As in the males the growth of the female rats deprived of the thyroid apparatus at 75 days of age in body length and body weight was less retarded than it was in rats similarly treated when 100 days old. In the females the tail length also participated in the lessened inhibition. The younger animals are more resistant to the growth inhibiting effects of thyroid deficiency. The analysis of this difference as made on the males will be given shortly.

2. The growth of the rats deprived of their parathyroid glands when 75 days of age in body weight and tail length was less retarded than was that of the rats similarly treated when 100 days old. The difference in the body length values was in the same direction but was too small to be significant of itself, though taken in connection with the other two values demonstrates that the younger females were more resistant to the growth inhibitory action of the toxemia induced by parathyroid deficiency than are the older ones. This response is the opposite from that obtaining in the males.

The figures in table 11 and charts 5 and 6 give the basis for a more detailed analysis of the growth in body weight of the various groups of females.

On chart 4 are plotted the values of the coefficient of variability on age. It shows that in the females, as in the males, the variability in body weight tends to be increased by thyroid and parathyroid deprivation. There do not seem to be any significant sex differences in body weight variability.

Chart 5 shows that the removal of the thyroid apparatus at 75 days of age does not produce as marked an immediate loss of weight as that produced by the removal at 100 days of age; a result similar in kind to that exhibited by the males. In addition the female rats thyro-parathyroidectomized when 75 days old showed a decided tendency to resume growth, a phenomenon not exhibited by the 100 day old series. It is possible that if the period of observation of the latter had been extended similar reaction would have been shown. When the growth curves of the thypar females and males of the 75 day old series are compared it becomes evident that the males were less retarded and recovered to a greater extent than the females. This difference was also exhibited in the 100 day old series.

From chart 6 it is seen that the loss of weight which immediately follows the removal of the parathyroids was more marked in the 75 day old rats than the 100 day old ones, a reaction similar to that obtaining in the males. Moreover the drop was less marked in the females of both series than in the males. In the 75 day old series, however, the recovery from the initial retrogression occurred sooner and appears to be more marked than was that in the 100 day old series. As in the males, so in the females, the

TABLE 11

Summary of the weights of the form groups of females at each period of measurement

DAY	75	82	89	96	103	110	117	124	131	138	145	150
Controls for thypars												
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Mean	117.3	133.0	135.8	143.7	147.9	153.8	159.5	162.5	166.1	172.0	175.2	178.5
S.D.	18.8	18.5	17.4	16.6	17.1	15.2	13.9	13.3	14.8	15.8	15.5	15.8
P.E.M.	4.0	3.9	3.7	3.5	3.6	3.3	3.0	2.9	3.2	3.4	3.3	3.4
C.V.	16.0	13.9	12.8	11.5	11.5	9.9	8.7	8.2	8.9	9.2	8.9	8.8
Thypars												
Mean	112.6	112.3	110.4	111.6	111.5	116.8	120.5	119.6	122.3	122.7	123.1	123.0
S.D.	25.5	24.4	32.0	24.0	24.6	29.0	28.4	28.1	31.1	31.5	33.3	32.9
P.E.M.	5.0	4.8	4.3	4.7	4.8	5.7	5.5	5.5	6.1	6.1	6.5	6.4
C.V.	22.7	21.7	19.9	21.5	22.1	24.9	23.6	23.5	25.5	25.6	27.1	26.8
Controls for parathys												
Mean	109.5	119.7	127.2	133.6	140.7	143.7	148.7	152.9	158.6	162.2	164.5	168.5
S.D.	12.8	14.1	16.4	17.0	19.3	18.0	20.3	20.6	20.2	21.4	22.1	23.3
P.E.M.	3.1	3.4	3.9	4.1	4.6	4.3	4.8	4.9	4.8	5.1	5.3	5.6
C.V.	11.7	11.8	12.9	12.7	13.7	12.6	13.6	13.5	12.7	13.2	13.4	13.8
Parathys												
Mean	114.4	109.0	112.3	116.6	120.1	122.9	129.2	135.4	134.7	136.2	134.2	132.9
S.D.	13.0	15.8	20.9	21.2	22.2	19.4	22.6	21.7	22.0	22.9	23.2	25.5
P.E.M.	2.8	3.4	4.5	4.5	4.7	4.1	4.8	4.6	4.7	4.9	5.0	5.4
C.V.	11.4	14.5	18.6	18.2	18.5	15.8	17.5	16.1	16.3	16.8	17.3	19.2

cumulative effects of the toxemia of parathyroid deficiency lead to a tendency to loss of weight at the later stages. From the qualitative point of view, then, the females of this series run a course parallel to that of the males in their growth in body weight subsequent to parathyroid removal. This is quite different from that obtaining in the thypars and is indicative of a difference in the type of metabolic disturbance.

Turning now to the quantitative analysis of these differences there will be found in table 6 the equations for the growth curves of the three groups

of female controls, and in table 12 the data for the intergroup comparison of the effects of the experimental procedures comparable to those of table 7.

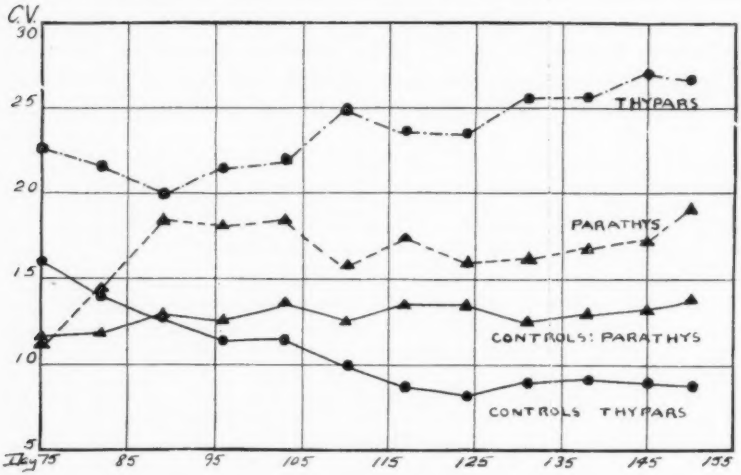

Chart 4. The coefficient of variability on age of the female thypars and parathys and their controls.

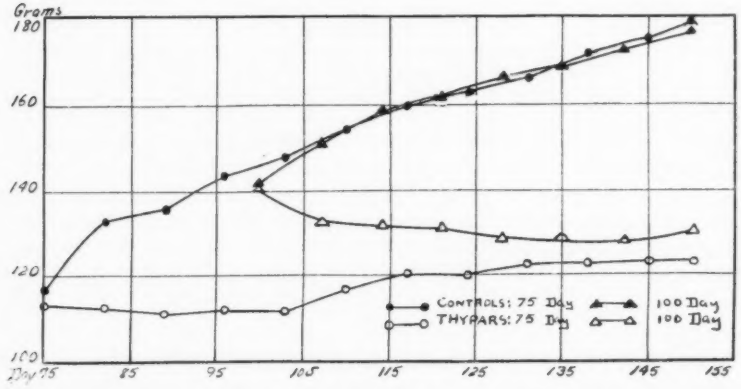

Chart 5. The weight on age of the female thypars and their controls.

The analysis of the body weight growth of the females. The loss of the thyroid apparatus and the parathyroids from female rats 75 and 100 days of age reduces the G.C. both as related to body weight and as related to age.

In both groups of parathys and in the thypars of the 75 day old series the average reduction was greater in the G.C. as a function of body weight than in the G.C. as a function of age. This is similar to the reaction in the males and is similarly interpretable.

The removal of the thyroid apparatus at 75 days of age does not cause as great a reduction in the G.C. either as related to weight or age, as does its removal from animals 100 days old. From the fact that the reduction in the G.C. as a function of age in the 100 day old thypars was practically the same as that of the G.C. as a function of body weight, and the fact that in the 75 day old series the weight-G.C. relation was cut to a greater degree than the age-G.C. relation, it is justifiable to conclude that the better growth of the younger thypars is due more to the resistance of the G.C. as related to the stage of development (age) than to the normally

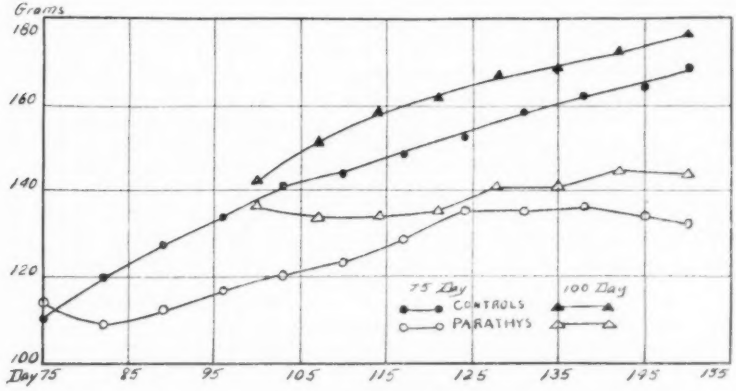

Chart 6. The weight on age of the female parathys and their controls

greater G.C. associated with body weight. It is to be understood that both factors participate.

It is improbable that the longer period of thyroid deficiency obtaining in the 75 day old series was a significant factor in their better growth. This is shown by the shorter duration of the negative G.C. (age) in this group and the tendency for the relative G.C. to decrease instead of increase in the later intervals.

The removal of the parathyroid glands from female rats 75 days of age causes a lesser reduction in the G.C. in both relations than does their removal from rats 100 days old. Since, however, the reduction in the G.C. as a function of age is of the same order of magnitude in both series, while the reduction in the G.C. as a function of body weight was proportionately greater with respect to that of the G.C.-age relation in the 100 day old

series than in the 75 day old series, it is evident that the better growth of the younger parathys is largely due to the G.C. as related to body weight (assimilatory metabolism) and that age (or stage of development) as such, has little to do with the degree of inhibitory response to the toxemia of the parathyroid deficiency.

TABLE 12

The expected and observed G.C. and their per cent difference. Females

AGE	75 DAY OLD SERIES						100 DAY OLD SERIES					
	Thypars			Parathys			Thypars			Parathys		
	Expected	Observed	O/E	Expected	Observed	O/E	Expected	Observed	O/E	Expected	Observed	O/E
G.C. as function of age												
75	1.21	−0.04	−3.3	1.27	−0.73	−57.5						
79	1.07	−0.16	−15.0	1.12	−0.06	−5.4						
86	0.88	−0.01	−1.1	0.91	0.50	54.9						
93	0.74	0.06	8.1	0.76	0.47	61.8						
100	0.63	0.39	61.9	0.64	0.37	57.8	0.92	−0.81	−88.0	0.90	−0.37	−41.1
107	0.54	0.54	100.0	0.55	0.56	101.8	0.70	−0.06	−8.6	0.70	−0.09	−12.9
114	0.47	0.13	27.7	0.48	0.74	154.2	0.55	−0.14	−25.5	0.55	0.10	18.2
121	0.41	0.13	31.7	0.42	0.24	57.1	0.44	−0.21	−47.7	0.44	0.59	134.1
128	0.36	0.17	47.2	0.37	0.06	16.2	0.36	−0.03	−8.3	0.36	0.04	11.1
135	0.32	0.04	12.5	0.33	−0.06	−18.2	0.30	−0.06	−20.0	0.30	0.41	136.7
142	0.29	0.01	3.4	0.29	−0.20	−69.0	0.26	0.20	76.9	0.26	−0.11	−42.3
Mean			24.8			32.2			−17.3			29.1
G.C. as function of body weight												
	1.34	−0.04	−3.0	1.14	−0.67	−58.8						
	1.35	−0.24	−17.8	1.29	0.43	33.3						
	1.40	0.16	11.4	1.19	0.54	45.4						
	1.37	−0.01	−0.7	1.08	0.43	39.8						
	1.37	0.67	48.9	0.99	0.33	33.3	0.95	−0.81	−85.3	1.07	−0.37	−34.6
	1.22	0.46	37.7	0.92	0.73	79.3	1.21	−0.06	−5.0	1.19	−0.09	−7.6
	1.13	−0.10	−8.8	0.79	0.69	87.3	1.23	−0.14	−11.4	1.16	0.10	8.6
	1.15	0.33	28.7	0.68	−0.07	−10.3	1.28	−0.21	−16.4	1.12	0.59	52.7
	1.08	0.04	3.7	0.69	0.16	23.2	1.36	−0.03	−2.2	0.94	0.04	4.3
	1.07	0.04	3.7	0.66	−0.21	−31.8	1.35	−0.06	−4.4	0.93	0.41	44.1
	1.06	−0.01	−0.9	0.70	−0.14	−20.0	1.37	0.20	14.6	0.81	−0.11	−13.6
Mean			9.4			20.1			−15.7			7.7

The nature of the data fails to support the idea that the better growth of the younger parathys can be attributed to the greater duration of the period of observation.

A comparison of the males with the females brings out certain qualitative and quantitative differences of interest.

In both series the reduction of the G.C. both as a function of age and as a function of body weight was greater in the female than in the male thypars. That is to say the females are the more dependent upon thyroid function for general growth than are the males.

The G.C. in both relations was cut to a lesser degree in the female thypars of the 75 day old series than in those of the 100 day old series with respect to the males. This indicates that the females approach more closely to the males with regard to the resistance to the inhibitory effects on growth of thyroid deficiency when the deprivation occurs at 75 days of age than when it occurs at 100 days of age.

The G.C. as a function of weight was cut proportionately more with respect to the G.C. as a function of age in the females thyro-parathyroidectomized at 75 days of age than in the males similarly treated. This shows that the lesser growth of the female thypars of this series was due more to the lesser resistance of the G.C. as a function of body weight than to the G.C. in its relation to age. It may be associated with the lesser normal G.C.-mass relation in the females. This supports the assumption that the greater the initial G.C. the greater the resistance to the growth-inhibiting properties of thyroid deficiency.

The females show a qualitative difference from the males in response to thyroid apparatus removal, in that in the former the age or stage of development of the animals seems to play the dominant rôle in the resistance to thyroid deprivation while in the latter the intensity of the assimilatory metabolism or G.C. as a function of body weight appears to be the more important.

From the charts it is evident that the course of growth of the male and female parathys is qualitatively the same in both and a cumulative effect of the toxemia is made clear by the regression of growth in the later intervals. From the tables it is evident that parathyroid deficiency causes a quantitative sex difference in growth, in that the G.C. both as a function of age and as a function of body weight is reduced to a greater degree in the males than in the females.

A qualitative sex difference with respect to age is also shown in the parathys by the fact that the reduction of the G.C. in both relations of the males of the 75 day old series was greater than that occurring in the 100 day old series, while with the females the opposite was the case. Furthermore it appears as if the age or state of development is more of a factor in the age differences of response in the males than in the females.

SUMMARY AND CONCLUSIONS

This paper contains a report of the growth in body length, body weight and tail length of male and female albino rats thyro-parathyroidectomized and parathyroidectomized at 75 days of age, and a comparison of the

response with that exhibited by rats similarly treated when 100 days old. Since a detailed discussion is to be found in the text, the general conclusions only will be given here.

The removal of the thyroid apparatus and the parathyroids at 75 days caused a retardation of gross growth in both males and females. The growth in body length was less retarded than was that of body weight. This differential response is the same as that exhibited by the thypars of the 100 day old series and is similarly interpreted.

In the thypar males the retardation of tail length growth was of the same order of magnitude as that of body length, in the females the retardation of body length growth was less than that of the tail.

The retardation of gross growth following thyroid removal at 75 days of age was less in both sexes than was that which followed the same procedure in rats 100 days old. As far as the growth in body weight is concerned it is probable that the greater resistance of the younger animals to the growth-inhibiting effects of thyroid deficiency is in large part attributable to the greater strength of the growth capacity at the earlier age. In the males this is referable rather more to the growth capacity associated with body mass, than to the age or state of development: in the females age is apparently a more potent factor.

The females are less resistant to the growth-inhibiting effects of thyroid deficiency. This was also found to be the case in the 100 day old series. The sex differences can be attributed in large part to the lesser strength of the growth capacity of the females at the time the glandular deficiency is initiated. It is probable that the growth capacity as related to body mass is proportionately more sensitive in the females.

The retardation of gross growth following parathyroid removal was greater in the males operated at 75 days of age than in those similarly treated when 100 days old. An analysis of the growth in body weight leads to the conclusion that the stage of development or age, and the greater interval of exposure to the toxemia of parathyroid deficiency are factors of importance.

The retardation of the gross growth of the parathyroidectomized females was less when the glands were removed at 75 days of age than when they were removed at 100 days. This better growth of the younger female rats is associated more with a greater resistance of the growth capacity as related to body mass, while age or stage of development is of minor importance.

Although the female rats parathyroidectomized when 75 days of age were less retarded in gross growth than were the males, the course of growth in body weight was practically the same for both sexes. This shows a qualitative sex similarity of response to the toxemia. The better growth of the females is to be attributed to the greater relative

resistance of the growth capacity as related to body mass, and the probability that age or stage of development is a factor of importance in the males. In both sexes a cumulative effect of the toxemia of parathyroid deficiency is evident.

While these analyses have been made on body weight observations made at weekly intervals, the conclusions derived therefrom are applicable in principle to the growth in body length and tail length.

These observations show that there are definite age and sex differences in the growth response of the albino rat to thyroid and parathyroid deficiencies; that the two experimental procedures produce qualitatively and quantitatively different results; and that there is a differential effect exerted upon body length, body weight and tail length.

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FACTORS INFLUENCING THE PRODUCTION OF INSULIN

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Received for publication November 28, 1923

While in certain early cases of diabetes mellitus no morphological changes in the pancreas can be demonstrated histologically, more advanced cases do show changes, and in certain types of diabetes these are of a purely degenerative character. This might suggest that in these latter cases the islets had suffered from prolonged overwork, that the metabolism of sugar throws a strain on them, and that an increased percentage of sugar in the blood may be a stimulation to insulin production. This view has been held by Allen and others for some time past, and the greatest proof of its validity is to be found in the fact that when the blood sugar of diabetic patients is maintained normal by dietetic treatment there is, in almost all cases, an increased tolerance due to the relief from overstrain.

In order to prove more concretely that an increased percentage of sugar in the blood stimulates the islet cells, the following experiments were carried out. The normal blood sugar was determined, after which dogs were anesthetized for thirty minutes. For the effect of ether administration on blood sugar see chart 1, figure I. After the period of anesthesia an intravenous injection of 2 grams of glucose per kilo of body weight (10 per cent glucose solution) were given. The blood sugar was followed until it reached a fairly constant level. A day or two later the same procedure was carried out with the exception that during the period of anesthesia the pancreas was removed. The time required for sugar injections in this series of experiments varied from four to seven minutes. For typical examples of the effect on the blood sugar see chart 1, figures III, IV, V, VI, VII and VIII.

It will be noted that when the pancreas is present the injection of glucose, *S*, was followed by a sharp rise in blood sugar, returning to the normal level in one and a half to two hours. Whereas the injection given immediately after pancreatectomy was followed by the same sharp rise; there was no return to normal, but the blood sugar remained at a high level. The ultimate lowering can be partially accounted for by the excretion of the sugar in the urine. Ether administration caused a less marked rise in the percentage of blood sugar when it was unaccompanied by operative procedure than when pancreatectomy was performed. Chart 1, figure II,

shows the gradual rise in blood sugar following pancreatectomy. This indicates that there is a continual secretion of insulin by the islet cells because when the islet cells were removed the percentage of sugar in the blood gradually increased. There is a variation in the appearance in the blood sugar curves following pancreatectomy and glucose injection, as seen by comparing figures IV, VI and VIII. It is to be noted that dog 15 received the sugar injections on two successive days with no other food, but with dogs 12 and 55 a day or two was allowed to intervene between the injections and during this time the dogs received their usual diet. Figures IX and X show the blood sugar curves following glucose administration, without anesthesia, one day and three days respectively after pancreatectomy.

These experiments show that when the pancreas is present the blood sugar rapidly returns to normal after the injection of sugar, whereas following the removal of the pancreas the blood sugar remains at a high level since an adequate amount of insulin cannot be produced in the absence of the pancreas.

In order to show that the production of insulin in response to sugar is in no way dependent on the nervous system, the following experiment was carried out. On April 27, dog 50 was anesthetized, abdomen opened, the tail portion of pancreas was separated from its mesenteric attachments, and the pancreas was stripped off the duodenum. The pancreas was then cut across at the level of the larger duct; care was taken to prevent damage to the pancreatic branch of the superior mesenteric artery and vein which enter the tip of the uncinate process. The remainder of the mesentery was divided. In this way a pedicle graft of about one-fourth of the pancreas was prepared. A stab incision was then made about 2 inches to the right of the original incision, and the subcutaneous tissues were separated to form a pocket for the future graft. The graft was scraped to remove its serous covering and to present a raw surface. It was then drawn through the small incision, and sutured in the subcutaneous pocket. On a diet of dog biscuit and lean meat the dog remained sugar-free, and blood sugar normal for twenty-one days. At the end of this time 2 grams of glucose per kilo of body weight were injected intravenously. The blood sugar was followed as before, and the curve was of normal character and returned to the normal level in two and a quarter hours. On May 19 the abdomen was reopened and the graft was found firmly attached in the subcutaneous tissues. The artery and vein of the pedicle were dissected free and carefully stripped bare and thoroughly painted with 2 per cent silver nitrate solution, so that any remaining nerve fibers would be destroyed. On the following day the same amount of sugar was given intravenously and it was found that the blood sugar curve was again normal in character reaching the normal level at the end of two hours. On May 23

the blood sugar was 0.134, on May 25, 0.109 and on May 28, 0.082. On May 29 the pedicle was ligated and divided. On June 5 blood sugar was 0.104. About the middle of August the dog was operated on by mistake for another experiment. The wound became infected and the dog died about September 1.

Having found that a nerve supply is not essential for the normal function of the islets cells, it was decided to investigate the effect of an altered blood supply. It had been observed that in the attempt to produce diabetes by *1*, injections of streptococci into the pancreas (dogs 7 and 8); *2*, injections of boiling water into the pancreatic arteries (dogs 2 and 6); *3*, x-ray of the exposed pancreas (dogs 10, 17, 31, 45 and 47), the dogs in no case became diabetic, but in some cases showed a subnormal blood sugar for three or four days following the operative procedure. The common feature in all these cases was a damaged pancreas and its consequent hyperemia.

In order to test this hypothesis it was decided to increase the blood supply to the pancreas experimentally by the local application of heat. This was done as follows. A dog was anesthetized, abdomen opened and the tail portion of the pancreas separated from its mesenteric attachments. The pancreas was then wrapped about with thin rubber tubing both ends of which protruded through the abdominal wound. In later experiments a glass coil with rubber connections was used since it is a better heat conductor. The remainder of the wound was closed and the margins were injected with a local anesthetic (2 per cent novocain). The dog was allowed to recover from the anesthesia and operation, and when the blood sugar had returned to normal the pancreas was heated by passing hot water through the tubes.

From chart 2, figure I, it will be seen that, although there was considerable variation in the blood sugar level from hour to hour following the application of the irrigation tubes, the blood sugar did not go below 0.101 in eleven hours whereas figures II and III, and figure I, chart 3, show that by irrigation the blood sugar may be reduced to a subnormal level. It will be noted in chart 3, figures I and II that when the blood sugar reached the subnormal level the application of cold water produced a marked rise.

The temperature of the water used for irrigation varied from 50° to 55°C. If higher temperatures were used an increase in blood sugar and vomiting occurred, and one case in which boiling water was used resulted in unconsciousness. It is evident that the higher temperature caused pain which raised the blood sugar. When water over 60°C. was applied the blood sugar lowering effect of the hyperemia was overbalanced by the blood sugar raising effect of pain.

When the suprarenals were tied off and the pancreas undamaged (dog 59) the blood sugar rose and returned to normal but did not go below it, chart 3, figure IV. When however, the suprarenals were tied off and the pan-

creas damaged by the application of irrigation tubes and thus made hyperemic, there was a progressive fall in blood sugar to 0.048 when the animal died, chart 3, figure III.

These experiments indicate that in dogs undergoing irrigation without removal of the suprarenals, the fall in blood sugar due to hyperemia might be in part counteracted by increased production of epinephrin. This would certainly have been the case when very hot solutions were used since there would be constant irradiation to the suprarenals and reflex pain.

CONCLUSIONS

1. There is a continual secretion of insulin by the islet cells because when the islet cells are removed the percentage of sugar in the blood is gradually increased.

2. When the percentage of sugar in the blood is suddenly increased the islet cells are stimulated to produce an adequate amount of insulin to reduce the blood sugar to its normal level.

3. The secretion of insulin does not necessarily depend on the nerve supply to the islet cells.

4. The hyperemia produced by the local application of moderate degrees of heat to the pancreas results in the lowering of the percentage of sugar in the blood.

5. There is a suggestion of a relationship between the blood sugar lowering effect of hyperemia and the excess production of insulin on one hand, and the blood sugar raising effect of pain and the excess production of epinephrin on the other hand.

We wish to express our sincere thanks to Prof. V. E. Henderson, by whose kindness the facilities were supplied which enabled these experiments to be carried out.

The expenses of this research were defrayed from a grant from the Carnegie Corporation Research Fund.

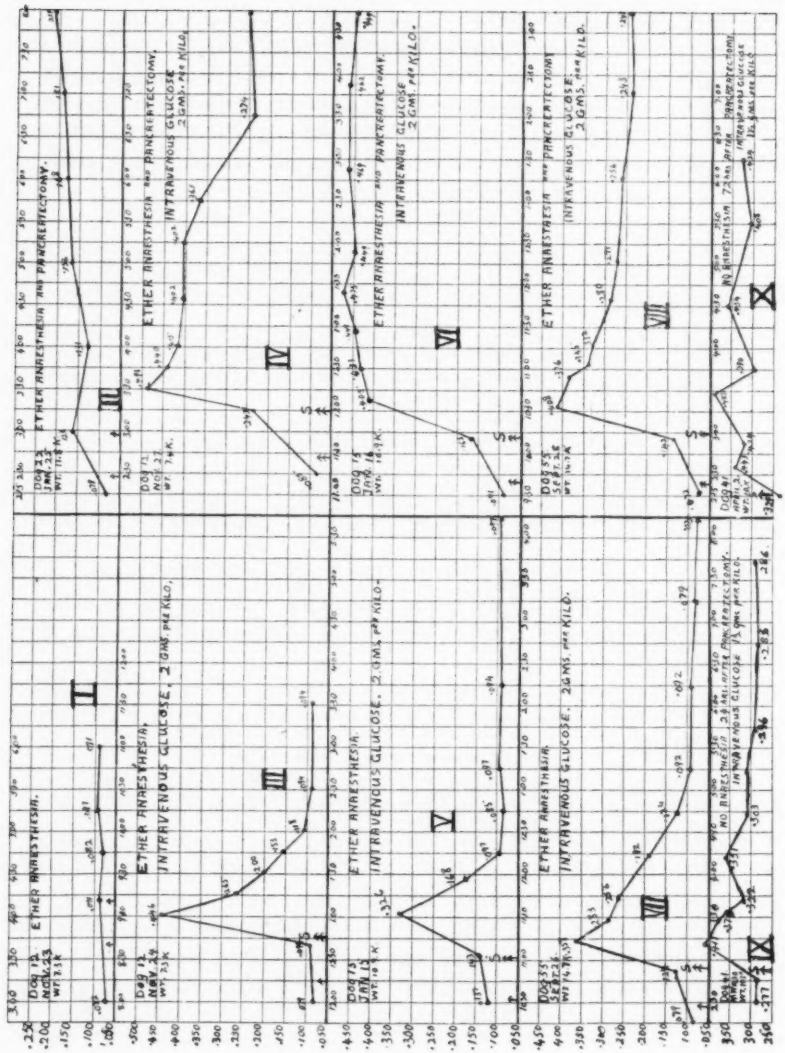
ETHER ANAESTHESIA.
INTRAVENOUS GLUCOSE. 2 GMS. PER KILO.
ETHER ANAESTHESIA AND PANCREATECTOMY.
INTRAVENOUS GLUCOSE 2 GMS. PER KILO.
NO ANAESTHESIA. 24 HRS. AFTER PANCREATECTOMY.
INTRAVENOUS GLUCOSE 2 GMS. PER KILO.

Chart 1

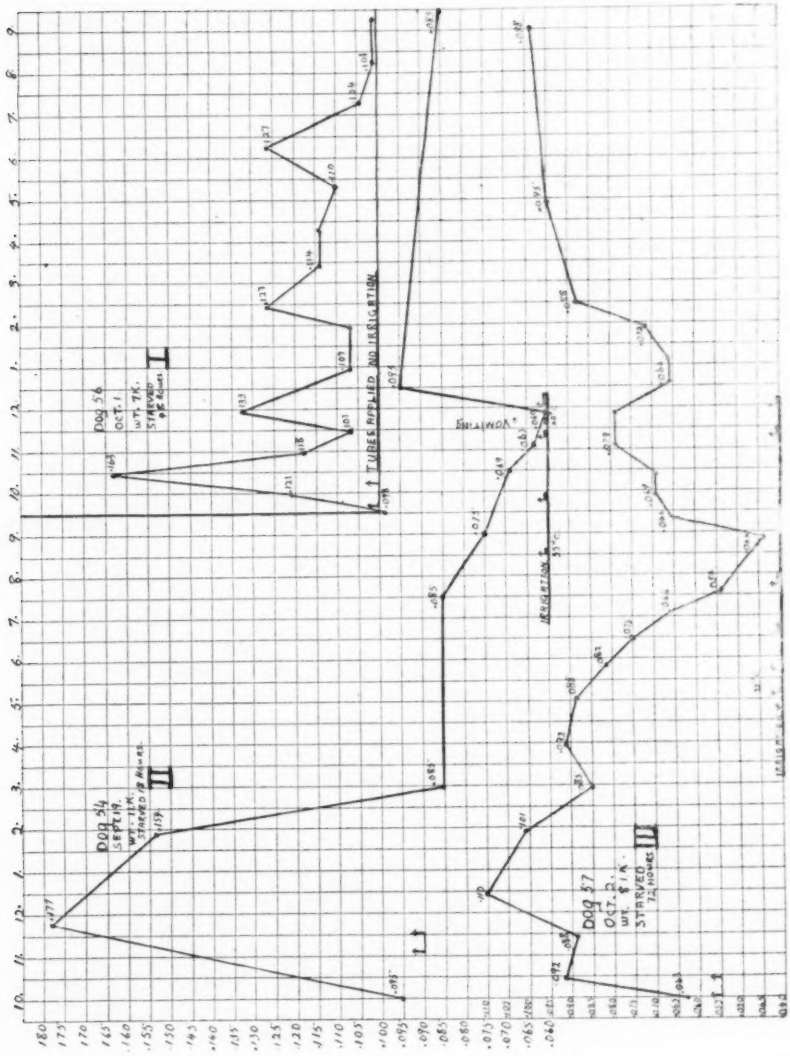
DOG 54
SEPT. 19.
WT. 11 K.
STARVED 18 HOURS.
II
DOG 56
OCT. 1
WT. 7 K.
STARVED
18 HOURS
I
TUBES REPLIED 2ND IRRIGATION
VOMITING
IRRIGATION
DOG 57
OCT. 2.
WT. 8 K.
STARVED
72 HOURS
III

Chart 2

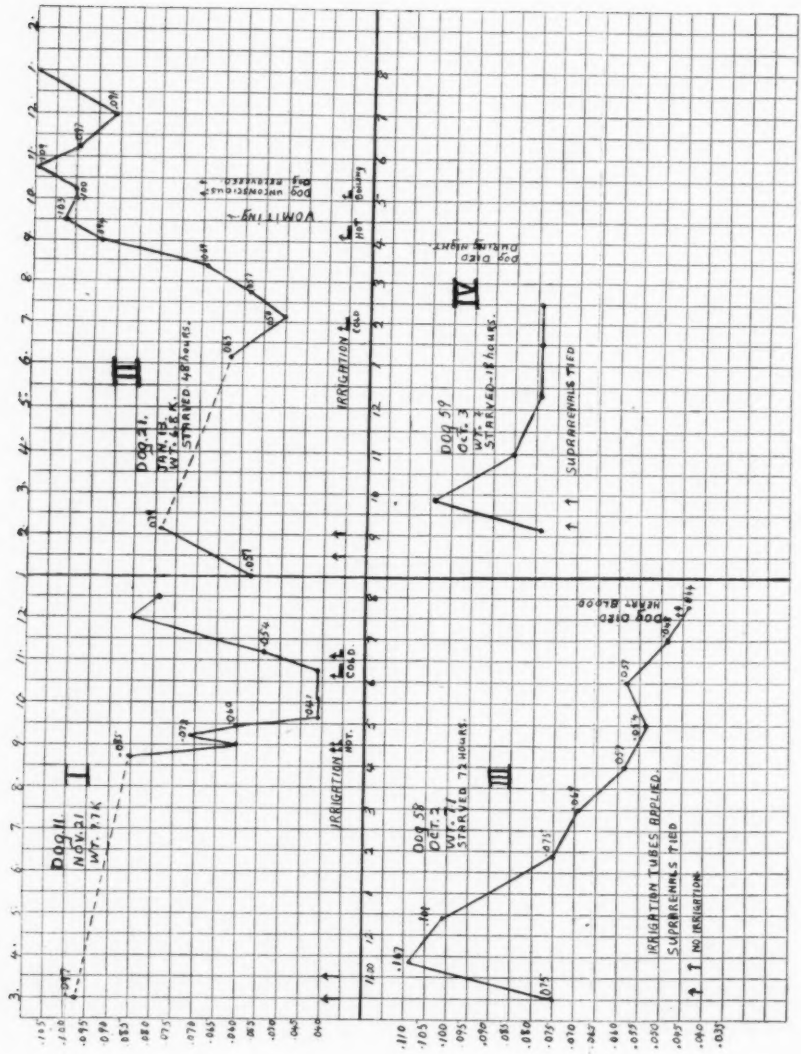
DOG II
NOV. 21
WT. 21K
I
DOG 21.
JAN. 18
WT. 6.8 K.
STARVED 48 hours
II
VOMITTING
DOG UNCONSCIOUS
DOG REVIVED
IRRIGATION
HOT.
COLD.
DOG 59
OCT. 3
WT. ?
STARVED 18 hours.
IV
SUPRARENALS TIED
DOG DIED DURING NIGHT
DOG 58
OCT. 2
WT. 11
STARVED 72 HOURS.
III
DOG DIED NEXT MORNING
IRRIGATION TUBES APPLIED.
SUPRARENALS TIED
NO IRRIGATION

Chart 3

CARDIAC ACCELERATION IN THE ABSENCE OF THE INHIBITORY CENTER

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Received for publication November 28, 1923

The question whether cardiac acceleration may occur independently of the inhibitory center is one which has been more or less discussed in the literature for the past twenty-five years or more. However, our views, and especially the views presented in a great many textbooks of physiology, are still somewhat hazy on the subject. In spite of a great many papers in the journals on the subject, it is still an open one, although many experiments have been performed to prove one view or the other. There are two possibilities under which cardiac acceleration may occur in an animal, since there are two mechanisms antagonistic to each other which are at work controlling the heart. While one tends to inhibit the heart and thus keep its rate down, the other tends to accelerate the heart and thus increase its rate. The question then arises which of these two antagonistically different mechanisms causes an increased heart rate? Theoretically, then, we may say that cardiac acceleration may be due to

1. A reflex depression of the inhibitory center or, in other words, inhibition of inhibition.

2. A positive activity of the accelerator center, reflexly or otherwise.

These two views are actually presented to us in the literature and each view has its adherents. Hunt (1) presents the view that cardiac acceleration may occur only through a depression of the vagus action. He claims that reflex cardiac acceleration is caused by inhibition of the cardio-inhibitory center and that he could find no evidence that the accelerator nerves are ever thrown into activity reflexly. He also claims that the most important function of the accelerator nerves seems to be connected with their tonic action; aside from this their function is obscure.

Gasser and Meek (2) claim that the acceleration of the heart at the beginning of voluntary exercise in the normal animal is chiefly due to the decrease in the tone of the cardio-inhibitory center. Their work leads them to believe that the accelerators are a factor of safety and that, in exercise, their action is superimposed on that of the vagi only in time of great need. Aside from this, these authors claim that their function is to maintain the level of the resting pulse.

Roy and Adami (3) and MacWilliam (4) also state that cardiac acceleration may occur only through a depression of the vagus function, and that the accelerators are in no way concerned with cardiac acceleration. A great many more cases might be cited where the conclusion has been reached that cardiac acceleration may occur only through a depression of the vagus function, but the few mentioned above will suffice for the present.

Hunt and other adherents of the first theory presented showed that cardiac acceleration may occur if a sensory nerve is stimulated when the vagi and the accelerators are intact but that no accleration could be shown after the vagi were sectioned and a sensory nerve stimulated. They also showed that, even when the heart was slowed by stimulation of the peripheral end of one of the cut vagi, no cardiac acceleration could be obtained if, at the same time, a sensory nerve was stimulated. Hunt (loc. cit.) also showed that if the accelerators were sectioned with the vagi intact, stimulation of a sensory nerve did give an accelerated heart rate. As a result of all these experiments Hunt and others inclined to the view that cardiac acceleration may occur only through an inhibition of the cardio-inhibitory center.

Opposed to this view is the second supposition that cardiac acceleration may be due to a positive activity of the accelerator nerves reflexly through the accelerator center. This view, too, has many adherents, and only a few of their results will be discussed here.

Bayliss (5) stimulated the depressor nerve in a rabbit after the vagi had been sectioned and obtained a definite increase in the rate of the heart. Since the vagi were no longer present, it would seem that this experimental result was contrary to the view proposed by Hunt and strengthens the view that cardiac acceleration may occur independently of the vagus center.

Hering (6) reaches the conclusion that cardiac acceleration after muscular exercise is dependent on the integrity of the accelerator nerves.

Hooker (7) in a series of experiments on the question of cardiac acceleration reaches the conclusion that there is positive evidence that reflex cardiac acceleration may occur independently of the cardio-inhibitory center. This author also claims that there is a physiological maximum rate of the heart which is usually reached after section of the vagi and beyond which it can only with difficulty be increased. He further states that, if such is the case, the limited amount of evidence in the literature favoring reflex cardiac acceleration through the accelerator center is easy of explanation.

Recently the papers of Wickwire (8), Yates (9), Winkin (10) and Tulgan (11) have brought out some further evidence on the functions of the cardiac nerves, and as a result it seemed advisable to reopen the question of cardiac acceleration.

Experimental procedure. All these experiments were done on cats. The laboratory procedure was ether anesthesia and tracheotomy. Blood pressure and heart rate were recorded by means of a mercury manometer connected to the left carotid artery. Records were made on a Hürthle kymograph. The time was recorded in seconds by a Jacquet chronograph. A signal magnet served as the base line for blood pressure and also indicated by a mark stimulation of a nerve or any other procedure. The heart rate was later computed in number of beats per 10-second intervals. In all cases a control tracing of blood pressure and heart rate was taken before any other procedure was attempted. The depth

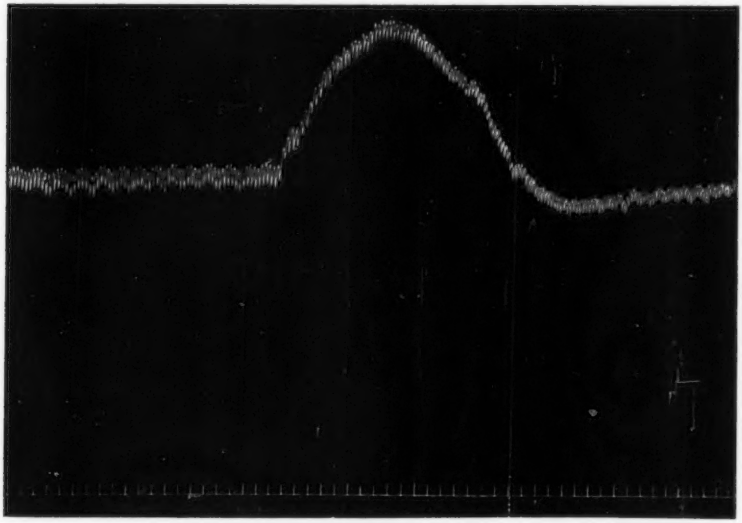

Fig. 1. Stimulation of the central end of the sciatic with the vagi and the accelerators intact.

of anesthesia was uniform throughout the experiment in each case. At all times a sufficient amount of ether was given to abolish all sense of pain in the animals experimented on.

I. STIMULATION OF THE SCIATIC NERVE WITH THE VAGI AND ACCELERATORS INTACT. After a normal tracing of blood pressure and heart rate was taken, the sciatic, and in some cases the brachial, nerve was divided and the central end stimulated with a medium tetanizing current. A definite rise in blood pressure and an increased heart rate were obtained in every case. Since the vagi and the accelerators were intact, it would be impossible to judge from these results whether cardiac acceleration in this case was due to inhibition of the vagi or to a positive activity of

the accelerators, but when the results obtained in this case are contrasted with the results obtained in the next series of experiments, the value of this experiment will be evident.

	RATE IN 10 SECONDS
Normal	32
Stimulation of the central end of the sciatic, vagi and accelerators intact	40

II. STIMULATION OF THE SCIATIC NERVE WITH THE VAGI INTACT, AND THE STELLATE GANGLIA REMOVED. In this series of experiments the central end of the sciatic nerve was stimulated after the stellate ganglia had been removed. The method used for the dissection of the stellates

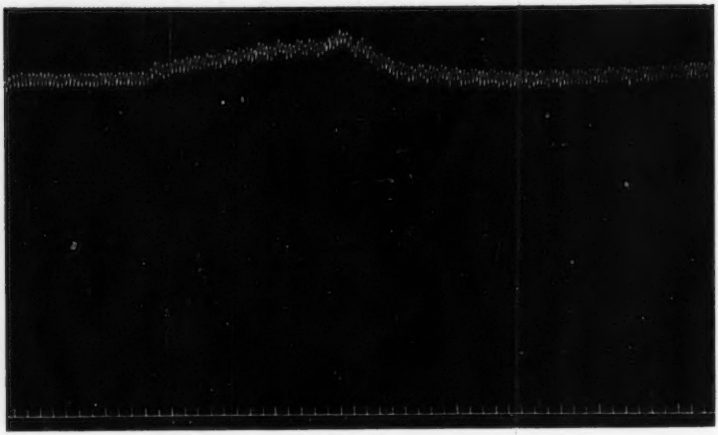

Fig. 2 Stimulation of the central end of the sciatic with the vagi intact and the accelerators cut.

has been described in a previous paper (11) and therefore need not be repeated here. The effect of the stimulation in this case caused a slightly increased blood pressure but there was no evidence of any increase in the heart rate. With the vagi still intact it is difficult to see why an increased heart rate should not be obtained if the theory that cardiac acceleration is due to an inhibition of the inhibitory nerves is correct. Since we do get an increased heart rate with the accelerators and the vagi intact, and do not get any increase in heart rate with the accelerators sectioned, it would seem more likely that an increase in heart rate in this case is due to the accelerators and not to the vagi.

	RATE IN 10 SECONDS
Normal rate after removal of the stellate ganglia	32
Stimulation of the central end of the sciatic, vagi intact, stellate ganglia removed	32

III. EFFECT OF STIMULATION OF A SENSORY NERVE WHILE AT THE SAME TIME STIMULATING THE PERIPHERAL END OF ONE OF THE CUT VAGI. If we assume with Hooker that the heart has a physiologically maximum rate which is obtained when the vagi are sectioned, and beyond which rate it is practically impossible to increase it, it is evident that after section of the vagi no increase in rate can be obtained on stimulation of a sensory nerve because the heart is already at its physiological maximum rate. If, however, we can slow the heart up somewhat and then stimulate a sensory nerve we should expect an increase in the heart rate to occur. This was actually done in the following manner. After the vagi had been sectioned, the peripheral end of one of the cut vagi was stimulated and the heart rate thus decreased. At the same time the central end of the cut vagus was also stimulated. The rate immediately increased and stayed higher until the stimulus was removed from the sensory nerve. Immediately the heart rate slowed up again, since the stimulation of the peripheral end of the vagus was continued throughout the period. The same experiment was done, using the central end of the sciatic instead of the central end of the vagus, with the same results.

It would seem from these results that cardiac acceleration may occur through the accelerator center provided the heart is not at its physiologically maximum rate under which circumstances no further acceleration can occur.

SUMMARY. At least 50 experiments were done on cats to determine whether cardiac acceleration is due to a decreased activity of the vagi or to a positive activity on the part of the acclerators. Representative tracings of the three methods of attacking this problem are given. From the results obtained it would seem that cardiac acceleration is due to a positive activity of the accelerator nerves and not wholly to a decreased activity of the vagi.

If a sensory nerve is stimulated, and in these experiments the central end of the vagus, the sciatic and the brachial were used, with the vagi and the accelerators intact, a definite increase in heart rate is observed. This may be due either to a positive activity of the accelerators reflexly through the accelerator center, or to a decreased activity of the vagi reflexly through the inhibitory center. If we assume that the heart rate is at its physiologically maximum rate when the yagi are sectioned, and that we cannot get any further increase on stimulation of a sensory nerve,

then with the vagi intact the heart is not beating at its maximum rate and consequently a definite increase in the rate should be observed on stimulation of a sensory nerve. This increase is actually obtained (fig. 1) and is, as I believe, due to a positive activity of the accelerators, as the next series of experiments shows.

In these experiments a sensory nerve was again stimulated but this time the stellate ganglia were removed previously, and the vagi were left

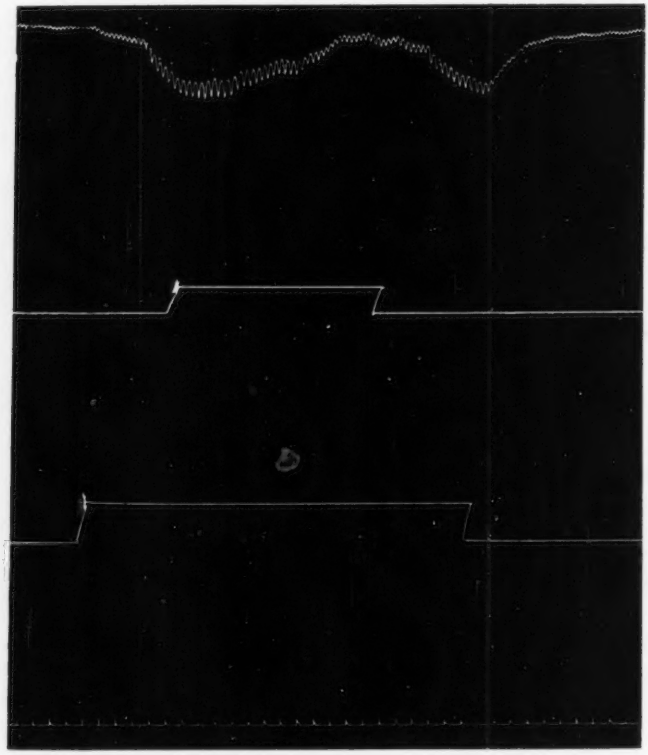

Fig. 3. Stimulation of the central end of the vagus during the period of stimulation of the peripheral end of the vagus.

intact. No increase in heart rate was ever observed. It is difficult to see why cardiac acceleration should occur as a result of inhibition of the vagi with the accelerators intact but why it should not occur with the accelerators removed. This experiment seems to prove that acceleration of the heart with all the cardiac nerves intact is due, not to an inhibition of the vagi, but to a positive activity of the accelerators.

The last series of experiments also proves that if the heart is not beating at its physiological maximum, a definite increase in its rate may be obtained when a sensory nerve is stimulated even when both of the vagi have been sectioned. The heart rate may be decreased by stimulation of the peripheral end of one of the cut vagi. If now the sciatic, the brachial, or the central end of the vagus be stimulated during the time the peripheral end of the vagus is stimulated a definite increase in heart rate is observed. Just as soon as the stimulus is removed from the sensory nerve the heart immediately slows up again. Reference to figure 3 will bring this point out.

One thing[1] seems to have been lost sight of in the discussion so far, or at least to have received insufficient emphasis in the literature. The normal heart rate of an animal rises when the vagi are sectioned, and falls when the accelerators are divided subsequently. The rate falls when the accelerators are divided and rises again when the vagi are sectioned afterward. Theoretically the final rate of the heart should be the same, whatever the order in which the nerves are eliminated. The rate of the heart when all the nerves are cut may be considered as its own autochthonus rate, dependent upon the intrinsic cardiac mechanism. This should be taken as the standard of comparison if we are to get a correct idea of the influences which regulate the heart rate. It should be obvious, also, when the facts are once presented, that inhibition of inhibition cannot, of itself, give rise to a rate any higher than that of the completely denervated heart. The limits of such a change would be that observed when the vagi are divided subsequent to removal of the stellates. It has been shown (8), (11), that the rate of the heart never becomes very high when the vagi are divided following the removal of the accelerators. If the rate of the heart becomes higher than that of the completely denervated heart, some other influence than mere inhibition of inhibition must be operative. The great increase of heart rate after section of the vagi with the accelerators intact can not be considered as due to inhibition of inhibition alone. The increase due to inhibition of inhibition alone would be only that difference between the rate of the heart as observed when all the nerves are intact and the rate which would prevail when all the nerves were cut, and only when the latter is the higher rate of the two.

A concrete example of this may be given. Suppose the observed rate of the heart to be 60. After section of the vagi it rises to 100. After section of the accelerators it falls again to 70.[2] Evidently, only the

[1] This was suggested by Prof. F. H. Pike, to whom I am very much indebted.

[2] Some observations on the rate of the heart of cats after complete elimination of all central nervous influences are given by Stewart, Journal of Experimental Medicine, 1906, viii, 320, and This Journal, 1907, xx, 407.

increase from 60 to 70 can be regarded as due to inhibition of inhibition. The increase from 70 to 100 must be regarded as due to the action of the accelerators. If the heart rate is 60 after section of the accelerators, the vagi being intact, and if it increases to 80 on stimulation of an afferent nerve, the increase from 70 to 80 can not be regarded, without further proof, as due to inhibition of inhibition alone. To regard this increase above 70 as due to inhibition of inhibition is to neglect the accelerator fibers in the vagus (12).

We may sum up the argument by saying that the increase in heart rate above that of the completely denervated heart cannot be regarded as due to inhibition of inhibition, but must be due to some accelerator influence. The clear recognition of these facts should help to clear up much of the confusion in the literature.

CONCLUSIONS

1. An increase in the heart rate may occur independently of the cardio-inhibitory center.

2. Provided the heart is not beating at its physiologically maximum rate, a definite increase in the rate may be obtained by stimulation of an afferent nerve after the vagi have been sectioned.

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A NOTE ON "FEMININE" BEHAVIOR IN ADULT MALE RATS

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Received for publication December 1, 1923

Upon rare occasions adult male rats have been observed to mount one another under ordinary conditions of cage confinement. This behavior occurs most frequently, however, when strange males are introduced into a cage of males, or when several males, still in a state of sexual excitement following prolonged copulation, are again brought together in their home cage.

Ordinarily a male thus mounted immediately effects his release from the copulatory clasp by a series of movements such as jumping forward, rearing upward, twisting aside, half turning the body or engaging in combat. Repeated mounting attempts, so far as the writer's limited observations go, do not eventually cause a male thus mounted to assume a pose or attitude characteristic of the receptive female or to modify in other ways his masculine behavior in the direction of femininity. Even in instances wherein a male has been repeatedly mounted by males that habitually dominate him in the home cage, resistance to the copulatory clasp persists.

Within the past year two cases of atypical behavior on the part of adult rats have been observed. Two males were discovered which did not resist the mounting attempts of other males. Furthermore, they responded to the latter's copulatory attempts in a manner characteristic of normal females in the receptive phase of the oestrual cycle. In view of the rarity of such behavior in what seemed to be normal males and because of the possible bearing it may have on reports of hermaphroditism experimentally produced in animals, these cases are herein reported. It is hoped that similar communications from other experimenters will serve to indicate the frequency with which such behavior occurs.

Case I. A medium-sized adult rat, weighing approximately 250 grams. Prior to the first bisexual behavior detected, this male had been used as a "tester" to determine whether female rats were in heat. His particular qualifications for this service were unusual sexual vigor and aggressiveness. Several litters were sired by him.

Males regularly employed as "testers" ordinarily become greatly excited in the course of an evening's work. Because of this excitement they frequently continue in copulatory attempts, one upon the other, when returned to their home cages.

Since such behavior had often been noted in other rats, no particular attention to it was given in the present instance until a chance observation revealed atypical behavior on the part of one male. It was noticed that one male was repeatedly mounted by two other males with which he was quartered and that he did not in turn attempt to mount either of them. Contrary to expectation, he did not resist the copulatory attempts of cage-mates; furthermore, he gave in response to their palpation of his sides a typical feminine response. He depressed his back in the lumbar region, elevated the coccygeal region, arched the tail, and threw back the head in a manner exactly duplicating the behavior of the receptive female in copulo. From three to four nights per week over a period of thirty days the behavior of this male was observed under various experimental conditions. Throughout that time he played the rôle of male when in the company of a receptive female, but in the presence of a sexually excited male permitted mounting and responded in a manner typical of a receptive female.

Eventually this male was sacrificed in order that his reproductive tract might be examined. Active spermatozoa in great numbers were found in the ductus deferentes and the testes. Nothing resembling ovarian tissue was discovered.

Case II. A large adult male weighing approximately 300 grams. His responses to receptive females were normal in pattern and in vigor. Evidence of ability to beget young was at hand. Prior to these observations he had been used as a "tester" of sexual receptivity of females. To the mounting attempts of other males he responded in a manner similar to that described in the foregoing case history. In the presence of receptive females he played the rôle of a normal, aggressive male, but immediately thereafter, when mounted by other males, responded in a manner typical of the receptive female. Systematic observations were carried on for three weeks with this male and in that time no variation from this dual form of sexual behavior was noticed. Unfortunately, during a temporary lapse of experimental work, this rat was lost without opportunity for autopsy. Hence nothing can be said concerning the reproductive apparatus beyond what may be inferred from his ability to beget young.

Feminization of male rats has been described by various investigators (1), (3), in connection with somatic changes resulting from ovarian transplantation. So far as one may ascertain from the published accounts of these experiments only partial transformation of masculine behavior was effected. A feminine "tail reflex" was added to the male's repertoire of responses, his attitude toward other males became less aggressive, or a solicitude for suckling young was acquired.

Such is the condition obtaining in the two cases herein reported. Responses which have hitherto been considered peculiar to the female,[1] *and to her alone*, were exhibited by males that seemed to be perfectly normal. Naturally the question has arisen as to whether the behavior of these two males was really *very* exceptional as the writer has hitherto been led to suspect. Has the sexual behavior of the male rat been studied with sufficient thoroughness to enable one to judge with what frequency the "feminine" behavior exhibited by the males herein described might be found

[1] For a more detailed account of the female's sexual behavior, see (4), pp. 101–105; (2), pp. 70–72.

in any random sampling of adult males? What per cent of male rats, if any, will exhibit under ordinary laboratory conditions, and apart from training or ovarian treatment, the feminine sexual responses which experimenters claim to have induced through the influence of ovarian transplants? What per cent of normal male rats can, with the aid of special environmental settings and modes of stimulation, be made to display elements of feminine sexual activity?

Until these questions are answered more satisfactorily than available data now permit, there is no adequate basis for harmonization and evaluation of the published accounts of hermaphroditic behavior experimentally produced in rats.

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PARADOXICAL PUPIL CONSTRICTION FOLLOWING LESIONS OF THE PROPRIOCEPTIVE PATHS

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Received for publication December 3, 1923

In 1905 Anderson (1) showed that after removal of the ciliary ganglion, or section of the short ciliary nerves, paradoxical constriction could be evoked in the homolateral pupil by asphyxiation, intravenous injections of 0.5 cc. of lactic acid, instillations of eserine pilocarpine, etc. Recently Byrne has shown that after somatic (2), (3), (4) and visceral (7) lesions involving the noci-ceptive (affective) system paradoxical dilatation, true and pseudo-, may be evoked by suitable means. The present paper shows the occurrence of paradoxical constriction after lesions of the proprioceptive (critical) system.

Method. This consisted of injuring *a*, one or more of the external ocular muscles of one eye; *b*, or one or more of the antigravity muscles on one side of the body; *c*, or one otic labyrinth; *d*, or one vagus nerve in the neck; and subsequently testing the sensitivity of the constrictor mechanism to miotic agents. For the daily tests it was found that conjunctival instillation, one drop in each eye of a 0.25 to 0.5 per cent solution of eserine sulphate, was the most serviceable agent. The possible defects of the instillation, such as inequality of absorption due to escape through the lachrymal duct, or at the outer or inner canthus, proptosis, etc., were fully appreciated at the outset and were effectively offset by extreme care in making the instillations, by frequent repetition of them over long periods, and by checking the results from time to time with intravenous injections of 0.5 cc. of lactic acid or of 1/8 molecular solution of Ca Cl_2. Anderson (1) had previously used the lactic acid and Auer and Meltzer (10) the Ca Cl_2 as mioties. The pupils were tested with eserine before subjecting the animals to experimental injury. The external ocular muscles in one set of cases were reached by inserting a tenotomy hook through an incision made into the conjunctival sac. In another set Anderson's method (1) was followed in which the orbit was reached through a Y-shaped incision the stem of the Y overlying the zygoma whilst the arms embraced the outer angle of the orbit. After much experimentation it was found that following injury of the orbicularis palpebrarum, or of its motor nerve, marked paradoxical constriction was

readily elicited. This precluded the use of this route and the first mode of approach was reverted to, care being taken to avoid over-stretching of the orbicularis palpebrarum. In control experiments the conjunctival membrane was incised and a tenotomy hook introduced and manipulated but without attempting to catch or strain the muscles. After these latter procedures paradoxical pupil constriction could not be elicited. The lesions were verified at autopsy. Wherever the skin was incised, as in making lesions of the antigravity muscles, an equalizing skin lesion was made at the corresponding site on the opposite side of the body. In making lesions of the masseter and temporal muscles care was taken to avoid injury of the branches of the 7th cranial nerve and after such operations care was taken to see that the orbicularis palpebrarum exhibited no functional impairment.

Experimental facts. Paradoxical constriction was evoked in the pupil on the side of injury after the following lesions: a, Subconjunctival (periocular) injections of a 60 per cent solution of alcohol (3 animals). Twenty-four hours after these injuries true paradoxical dilatation phenomena were elicited. b, Mechanical injury (stretching or section) of the external ocular muscles through an incision made in the conjunctival membrane, viz., *internal rectus* alone, 13 times (fig. 1); *inferior rectus*, alone, once; *external rectus*, alone, twice; combination of *internal* and *superior rectus*, 9 times; combination of *superior rectus*, *internal rectus* and *superior oblique*, once; *right internal rectus* plus an equalizing incision in the *left* conjunctival membrane, once; *superior rectus* alone, once; combination of *superior, inferior and internal rectus*, once; *right internal and superior rectus* plus an equalizing incision through the left conjunctival membrane, once. c, Mechanical injury (stretching, section, etc.) of the *orbicularis palpebrarum* or of its *motor nerve supply* (5 animals). d, Cauterization of *one posterior funiculus* at level of root C V, in 1 animal (fig. 3). e, Section or injury of one vagus nerve in the neck. Eserine instillations failed to evoke paradoxical constriction after simple incision through the conjunctival membrane of one eye (2 animals) and after conjunctival incision and the introduction and manipulation of a tenotomy hook without injury of the orbicularis palpebrarum, or of the eyeball, or of the muscles attached to it.

Following injury of the *antigravity muscles* paradoxical constriction was evoked by eserine instillations, etc., as follows: a, In the *homolateral pupil* after *1, tenotomy of the triceps cubiti* (4 animals); *2, hemitransection* of spinal cord between roots C VI and VII (1 animal); *3, section of both cervical sympathetic nerves* plus tenotomy of *triceps cubiti* (3 animals); *4, spinal cord transection between roots C V and VI* plus crushing of one brachial plexus (1 animal); *5*, severance of attachment of *cervico-occipital muscles* on one side (3 animals); *6*, injection of 95 per cent alcohol

Fig. 1.

Fig. 2.

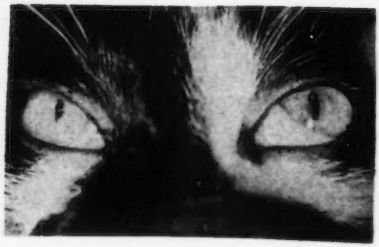

Fig. 3.

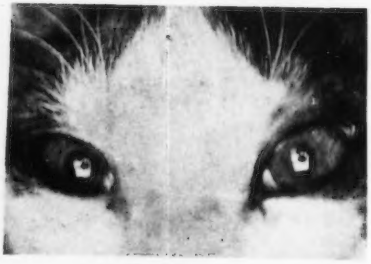

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 1. Paradoxical constriction (pilocarpine) in left eye 15th day after cutting left internal rectus; persisted after decerebration and section of both vago-sympathetic nerves.

Fig. 2. Paradoxical constriction (eserine, ether) in right eye 24th day after cutting right cervical sympathetic and left vago-sympathetic nerves and crushing left sciatic.

Fig. 3. Paradoxical constriction (eserine) in right eye 21st day after cauterizing right posterior funiculus at level of root C v.

Fig. 4. Paradoxical constriction (pilocarpine) in left eye 14th day after excising right patella.

Fig. 5. Paradoxical constriction (eserine) in left eye 7th day after transecting spinal cord between roots C V and VI and crushing right sciatic nerve; one hour after death.

Fig. 6. Paradoxical constriction (eserine, ether) in right eye 3 days after destruction of auditory portion (mainly) of right otic labyrinth.

Protocol. Cat 30. Female, adult, non-pregnant

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
	a.m.					
	8:20	June 7, 1923	3.0	3.0	1.0	1.0
	8:20½	Eserine sulphate solution 0.5 per cent 1 drop in each eye				
	9:20		1.0	0.5	0.5	0.5
	9:20½	Ether				
	9:32		5.0	5.5	2.5	2.5
	9:32½	Adrenalin mii intravenously	13.5	14.0	0.0	0.0
	9:50	Cut right cervical sympathetic and left vago-sympathetic nerves; crushed left sciatic				
2nd	8:44½	Pseudo-paradoxical dilatation right eye	3.0	2.5	3.0	3.0
	8:45	Eserine 0.5 per cent solution 1 drop in each eye				
	9:09	Paradoxical constriction in right eye	0.5	1.0	5.0	5.0
3rd to 10th		Tests made daily				
11th	9 14		2.5	3.0	2.5	2.0
	9:14½	Ether				
	9:25	Before adrenalin	3.0	3.5	3.0	3.0
	9:25½	Adrenalin mii intravenously; paradoxical dilatation absent in right eye	11.0	12.0	0.0	0.0
	9:31½		3.0	3.5	3.0	3.0
	9:32	Eserine 0.5 per cent solution drop 1 in each eye				
	9:53	Paradoxical constriction in right eye	0.5	1.0	2.5	2.0
24th	9:39½		2.0	2.5	3.0	2.5
	9:40	Eserine 0.25 per cent solution 1 drop in each eye				
	10:21	Paradoxical constriction in right eye	0.5	1.5	3.0	3.0
	10:38	Ether				
	10:55	Wink reflex absent; paradoxical constriction in right eye, figure 2	3.0	9.0	2.5	2.5
	11:20		0.5	1.5	1.0	1.0
	11:20½	Adrenalin mii intravenously; paradoxical dilatation absent in right eye	9.0	10.0	0.0	0.0
	11:36	Stimulation of right and left vago-sympathetic nerves proximal (caudad) of the site of section elicited no direct response in the pupil with the coil at a separation of 9.0, 6.0 and 4.0 cm.				
	11:52	Killed animal				

into cervico-occipital muscles on one side (1 animal); *7*, severance of insertion of temporal muscle on one side into deep surface and apex of the coronoid process of the mandible (1 animal); *8*, severance of insertion of masseter muscles into lateral surface of ramus and angle of mandible (1 animal). b, In the *contralateral pupil* after *1, excision of patella* and severance of aponeurosis, ligamentous extensions, etc., of tendon of *quadriceps extension cruris* in 2 animals (fig. 4); *2*, section of tendon of quadriceps extensor cruris and of tendo Achillis of one side (2animals); cord transection between roots C V and V1 and roots L 111 and 1V did not abolish the paradoxical constriction; *3, cord transection between roots C V1–V11* plus *tenotomy of quadriceps extensor cruris* (1 animal); *4, section of one tendo Achillis* (1 animal).

Contralateral phenomena were also observed after cord transection between roots C V and V1 plus crushing of one sciatic nerve (fig. 5) and after cutting right cervical sympathetic and left vago-sympathetic nerves and crushing left sciatic (fig. 2). After destruction of the otic labyrinth (5 animals) the homolateral constriction evoked by eserine and asphyxia was only less marked (fig. 6) than that observed after removal of the ciliary ganglion (3 animals). In two animals in which the labyrinthine injury was found to be confined chiefly to the auditory (cochlear) mechanisms marked paradoxical constriction was elicited.

Discussion and summary. True paradoxical dilatation following lesions of the afferent paths is preceded by an incubation or latency period averaging from 6 to 12 days depending on the nature and site of the lesion (4). Pseudo-paradoxical dilatation following lesions of the afferent paths seems to have no incubation period although the phenomenon tends to recede during the true paradoxical period. Paradoxical constriction, as encountered in the present studies, seems also to have no true incubation period. At times the phenomenon was absent for a day or so after operations on and about the eye but here the failure of eserine or pilocarpine instillations to evoke it was probably the result of local damage to the blood vessels and lymphatics which prevented the drugs from reaching the portion of the constrictor mechanism hypersensitive to eserine. Following removal of one ciliary ganglion eserine instillations failed to evoke paradoxical constriction during the first 24 hours after the operation although pilocarpine instillations readily did so. On the second day eserine evoked marked paradoxical constriction. These observations corroborate those of Anderson (1).

In true paradoxical dilatation following afferent path lesions the hypersensitivity of the dilator effector mechanisms is fundamentally determined by hypo-function of the injured primary neurones of the nociceptive (affective) system. This hypo-function is counterbalanced in the pre-paradoxical period by *hyper-function* of some slightly injured (over-

stimulated) noci-ceptive neurones. After the lapse of 8 or 10 days the algebraic sum of the centripetal neural discharges from the hyper- and hypo-functioning primary noci-ceptive neurones represents hypo- rather than hyper-function. This is the fundamental factor in the mechanism of true paradoxical dilatation following afferent path lesions (4). In the present studies function was suspended in the primary sensory neurones of the proprioceptive (critical) system by making it impossible to develop the adequate stimulus (pressure) in certain muscles by cutting, stretching or otherwise injuring their fibers, tendons and aponeurotic attachments. Depending on the care and thoroughness with which the lesions were made the suspension of function in the proprioceptive neurones was followed by immediate sensitization of the constrictor mechanism. Complications, however, of necessity arose from the inevitable involvement, to a greater or less extent, of the noci-ceptive system. With an understanding, however, of the mechanism and time relations, and above all of the immediate excitants (excitement of any kind) of pseudo-paradoxical dilatation, the sensitizing effects of hypo-function of the primary proprioceptive neurones upon the constrictor mechanism can be studied quite satisfactorily.

The constant relationship of paradoxical constriction to the lesions was repeatedly verified by the disappearance of the phenomenon as functional recovery of the injured muscles or tendons took place; by the actual findings on re-dissection in vivo and at autopsy; and by the constant reappearance of the phenomenon when, after healing, the muscle was again injured or incapacitated. The failure to evoke the phenomenon after mere incision into the conjunctival membrane, with and without the introduction of a tenotomy hook, points to injury of the ocular muscles as the significant factor in the mechanism. Inflammation confined to the conjunctiva does not induce the phenomenon. Infections, however, of severe grade involving the nerves and ethmoidal cells very readily lead to involvement of the long and short ciliary nerves with consequent development of hypersensitiveness of the dilator and constrictor effector mechanisms. In these cases, however, the paradoxical dilatation phenomena can be differentiated from the corresponding phenomena following lesion of the afferent paths by their short period of incubation (24 hours) and by the readiness with which they can be elicited, e.g., by instillations of adrenalin into the conjunctival sac, hypodermic injection of m xxv of a 1:000 solution of adrenalin and by very small amounts of very dilute solutions of adrenalin (1 cc. of 1:60,000) intravenously.

In the animals subjected to subconjunctival (periocular) injections of alcohol, adrenalin evoked true paradoxical dilatation in the injured eye 24 hours after the operation. This pointed conclusively to injury of the long ciliary nerves as the lesion responsible for the paradoxical dilatation.

Taken in conjunction with the moderate degree of pupil dilatation present in the resting injured eye, and the marked constriction evoked by eserine, it also points to injury of the short ciliary nerves as the lesion responsible for the paradoxical constriction demonstrable in the injured eye on the 2nd or 3rd day following the operation. In this type of injury the lesions responsible for the paradoxical dilator and constrictor phenomena are located mainly if not exclusively in the efferent pathways, i.e., in the long and short ciliary nerves. Of interest, however, is the fact previously observed by Anderson (1) that it is possible to have the dilator and constrictor effectors of the same eye simultaneously hypersensitive to their appropriate activators. Compare the animals in which lesions were made on the otic labyrinths. In these the post-ganglionic sympathetic paths were injured in their course through the bulla tympani giving marked true paradoxical dilatation phenomena which, however, did not interfere with the demonstration of the marked paradoxical constriction phenomena due to lesion of the labyrinthine proprioceptive mechanisms. The marked paradoxical constrictor effects obtained after lesions of the labyrinth point to the auditory nerve proper no less than the vestibular nerve elements as representing marked condensations of critical mechanisms comparable to the condensations of efferent dilator paths found in cervical sympathetic nerve. The extra-ocular muscles manifestly contain numerous proprioceptive elements. Compare the mechanism of accommodation and the relation it bears to equilibration and spatial orientation. Compare also Tozer and Sherrington (8) who demonstrated the existence of proprioceptive elements in the 3rd, 4th and 6th cranial nerves of the monkey.

In the lesions made upon the orbicularis palpebrarum and external ocular muscles no less than in those made upon the antigravity muscles it was evident that failure to develop the adequate stimulus (pressure) for the proprioceptive neurones distributed to the injured muscles was the fundamental factor in the mechanism. In the animals in which the phenomenon appeared after section of both cervical sympathetics and crushing of one sciatic nerve it was evident that the mechanism consisted of suspension or impairment of function in the proprioceptive paths passing to the brain and there impinging presumably on the constrictor center in the oculo-motor nucleus. This, however, only represents part of the mechanism in animals with uninjured cervical sympathetics. In the animals in which the spinal cord had been transected between roots C V and V1 and one sciatic nerve crushed the appearance of paradoxical constriction in the contralateral eye showed clearly that in the intact animal some of the proprioceptive impulses streaming cephalwards from the lower extremities passed to the head by other paths than those lying in the cervical portion of the spinal cord. Compare

Byrne (4) who found that pseudo-paradoxical dilatation following lesion of one sciatic nerve was only partially abolished by transection of the cervical spinal cord but was completely abolished by transection of the cord at the level of L1. As the hypersensitiveness of the constrictor effector mechanisms which had appeared after excision of one patella was not abolished after spinal cord transection between roots L III and IV, and between roots C V and VI, nor after removal of the ciliary ganglion, section of the short ciliary nerves, or instillations of homatropine, it seems that as in the case of true paradoxical pupil dilatation the hypersensitiveness following the lesions studied in this paper was confined to the constrictor effector mechanisms in the iris these mechanisms for the purposes of this paper being taken to include not only the myoneural junctions but the terminal nerve nets as well. The mechanism, therefore, of paradoxical constriction following such lesions as crushing of one sciatic nerve, or section of the tendons of the antigravity muscles of one hind limb, consists of interruption or impairment of the flow of proprioceptive impulses which reach the constrictor center in the oculomotor nucleus mainly but not exclusively through the spinal cord. In the case of the orbicularis palpebrarum and external ocular muscles the proprioceptive paths involved in the mechanism pass probably via the oculo-motor nerve or possibly through one or more divisions of the 5th cranial nerve.

As the paradoxical constriction encountered in these studies was not abolished by removal of the ciliary ganglion, spinal cord transection at various levels nor by decerebration a la Sherrington the proximate factor in the mechanism must be sought in the hypersensitiveness of the constrictor effector mechanisms, the remote fundamental factor being hypofunction of the primary proprioceptive neurones. The influence of injury of the afferent or efferent nerves supplying somatic (1), (3) or visceral (7) organs and of lesions of the afferent and efferent paths in the brain and spinal cord, in determining paradoxical hyper-function in the related pupil effector mechanisms has important clinical significance. There is evidence, experimental and clinical, that injury (mechanical, chemical, thermal, etc.) of the related afferent or efferent nerves may induce paradoxical hyper-functioning in almost any of the effector mechanisms of the body whether these be motor, inhibitory or secretory (including the endocrine glands). Compare the hyper-reflexia attending pyramidal tract lesions and that attending returned sensibility in the affective (noci-ceptive) system in the early regeneration period following lesions of the afferent nerves (6). Compare further the studies of Elliott (9) who has shown that paradoxical hyper-functioning (increased irritability to adrenalin) appears in plain muscle whenever it is denervated (decentralized). Hitherto the rôle of the nervous system has

been practically ignored in the mechanism of that complex of disordered function known as disease. The present studies seem to point to a promising field for those interested in the mechanistic interpretation of symptoms in disease. Of interest to clinicians also is the remoteness of the site of occurrence of the paradoxical hyper-function from the site of the lesions, and the fact that paradoxical hyper-functioning may be present simultaneously in closely related antagonistic effector mechanisms. Throughout the entire body the two great afferent systems, viz., the affective (noci-ceptive) and the critical (which includes the proprioceptive) function side by side in a coöperation of antagonism each, within certain limits of stimulation, controlling and modifying the action of the other and thereby determining in any given instance the type of response made to any particular stimulus. Compare Byrne (5), (6). Under ordinary conditions of contact with the environment no stimulus is purely affective or critical each affective or critical stimulus making appeal, actual or potential, to both affective (noci-ceptive) and critical (proprioceptive) mechanisms. The response, therefore, always represents the algebraic sum of the conflicting afferent elements and corresponds in type with one or other of the two fundamental modes of protection, viz., *instinctive* (imperative) or *cognitive* (selective) the former mediated mainly by the affective, the latter mainly by the critical system. In the spinal animal the *instinctive* type of response is fairly represented in the flexion reflex (noci-ceptive) and the cognitive type in the extensor thrust (proprioceptive) the former representing the fundamental primary element in the more complex noci-ceptive reactions having pain, fear, and the coarser emotions as their psychic adjuncts, the latter the fundamental primary element in spatial adaptations (equilibration, spatial orientation) underlying the more complex psychic elaborations incidental to cognitive defense reactions, viz., comparison, judgment, memory, will, etc. It seems, therefore, that besides furnishing the means for cognitive as opposed to instinctive modes of protective adjustment, the coöperation of antagonism of the two great afferent systems also furnishes the physiological basis of ordered sensation and psychic development.

CONCLUSIONS

1. After injury of the orbicularis palpebrarum, of the external ocular muscles, or of the antigravity muscles the constrictor effectors of the related pupil become hypersensitive to miotic drugs and other forms of stimulation, e.g., asphyxia, death, etc.

2. The constrictor hypersensitiveness is due to suspension of function of the primary neurones of the proprioceptive system supplying the injured muscles and consequent diminution of the flow of efferent constrictor impulses normally conditioned by the impingement of proprio-

ceptive impulses from the periphery upon the constrictor center in the oculo-motor nucleus.

3. Paradoxical pupil constriction seems to have no incubation or latent period, in this respect resembling pseudo-paradoxical dilatation.

4. As in the case of pseudo- and true paradoxical dilatation lesions in one hind quarter of the body are followed by paradoxical constriction in the contralateral pupil whereas lesions in one fore quarter, or of one side of the head and neck, are followed by paradoxical constriction in the homolateral pupil.

5. The mechanism of paradoxical constriction after crushing of one sciatic nerve consists in part of failure of afferent constrictor impulses that normally reach the constrictor center in the oculo-motor nucleus through paths other than those lying in the cervical portion of the spinal cord.

6. The efferent systems controlling pupillary movement have intimate functional relations with the two great subdivisions of the afferent system of nerves the constrictor mechanism being under the direct activating influence of the critical (proprioceptive) system and the dilator mechanism under that of the affective (noci-ceptive) system.

7. The influence of somatic and visceral (including cerebrospinal) lesions affecting the efferent paths in determining paradoxical hyperfunction of the effector mechanisms (motor, inhibitory and secretory including the endocrine glands) throughout the body has important clinical significance in the physiological interpretation of the symptoms of disease.

8. In the auditory as well as the vestibular portion of the eighth cranial nerve are found marked condensations of critical (proprioceptive) elements.

9. In the mechanisms of pupillary movement, as elsewhere throughout the body, the primary neurones of the affective (noci-ceptive) and critical (proprioceptive) systems function in a coöperation of antagonism which supplies the basis not only for cognitive (selective) as opposed to instinctive (imperative) modes of protective adjustment but also that for ordered sensation and psychic development.

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TEMPERATURE RECORDS OF ALASKA FUR SEALS

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Received for publication December 4, 1923

During a sojourn of several years on the Pribilof Islands, Alaska, opportunity occasionally occurred to secure temperature records of fur seals. Because the subject has an important bearing upon the commerical operation of the Government's industry of securing the furs of these animals, the data would seem to be of sufficient value for publication.

Apparently the only published observations on the subject were made by M. C. Marsh in 1906. He gave a table showing the heart, rectum and throat temperatures of eight animals which were as follows, summarized: heart, 39° to 41°C.; rectum, 37.5° to 43°C.; throat, 38.5° to 41°C. He also stated that the normal temperature was 37.5°C. although that is not the average of his figures.[1]

On June 29, 1918, heart temperatures were taken of twenty-five seals on the village killing ground of St. Paul Island. The measurements in these as in other cases mentioned herein were taken on the killing fields and the animals were males which had been driven for commercial purposes. This day was cool and dry. Seals had had ample time to cool from any overheating due to the exertion of the drive. They were kept in cold pond water until a few moments before the killing. Temperatures varied from 99.4°F. to 103.10°F. with an average 101.4°. The higher temperatures were recorded during the latter part of the work as the day became slightly warmer. The very noticeable increase, however, was apparently due to the excitement of the animals in pods or groups prior to the slaughtering operations rather than to atmospheric change. The ages varied from three to five years.

On July 3, 1918, heart temperatures of thirty-nine seals were taken on Zapadni killing ground, St. Paul Island. The range was from 101°F. to 106.6°F. with an average of 103.5°F. In this case the morning was hot with the sun shining brightly. No water was available for cooling. The seals

[1] Marsh's observations are on p. 364 of a complication of documents published in 1914 by the Government and entitled "Appendix A to Hearings before House Committee on Expenditures in the Department of Commerce and Labor, H. R. No. 73, 62nd. Cong. 1st. Sess." It is a rare, an almost inaccessible book except in the largest libraries.

were "panting" (breathing through their mouths) and in the pods fanned themselves with their hind flippers. Work of killing was dismissed before it had progressed far because of the dangers attending over-heating. The drive was turned back into the sea. One animal (a three-year-old) did become over-heated and was killed when in convulsions. Its temperature was 108.9°F. Upon examination it was found that the fur had loosened and could be easily plucked with the fingers.

On July 17, 1918, at North East Point, St. Paul Island, heart temperatures were taken of seven seals in the early morning. The day was cool and foggy and the animals had been kept for some time prior to killing in cold pond water. Temperatures ranged from 99.4°F. to 102.4°F. with an average of 101.2°F.

The deductions drawn from the observations made in these killings were checked on numerous other occasions. Some of the skins of animals measured were tagged and followed through subsequent operations until the final dressed and dyed fur was ready for sale and observations made upon them by Mr. William P. Zschorna are embodied in the following conclusions.

1. The normal temperatures of bachelor seals as received on the killing fields in a thoroughly cooled condition are about 101°F. These yield the best commercial furs.

2. When the temperature rises above 103°F. the animals display the heated condition by panting, and fanning with the flippers. Inferior furs result when taken under these circumstances.

3. Skins taken from animals which have died from over-heating (108.9° F.) or were taken at a temperature above 105°F. are of relatively small value.

Every possible precaution is taken in the industry to avoid over-heating or excessive exertion of the animals but conditions are such that these cannot as yet be wholly eliminated. Certain changes and improvements are necessary before the difficulties can be entirely overcome. The distance the animals are forced to travel overland between the hauling grounds and killing field must be shortened materially in some cases. This can only be accomplished through the construction of a system of roads over which the skins can be hauled to the settlements.

Natural ponds are not always available adjoining suitable areas to serve as killing fields. In these cases small reservoirs are urgently needed to keep the animals cool.

THE PHYSIOLOGICAL ACTION OF IONIZING RADIATIONS

I. Evidence for Ionization by β-Radiation

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Received for publication December 6, 1923

The general tendency of radiations to ionize matter has made it natural to assume that the physiological effects of radium and x-rays are due to the ionization of certain constituents of protoplasm. This view has been suggested repeatedly and is formulated as a definite theory by Joly (1) and Richards and Woodward (2). Only the most vague analogies between physical and physiological phenomena have been brought forward in support of the view. The most important fact in its favor is that in the case of chemical changes occurring in gases, a close relationship exists between the number of molecules undergoing formation and the number of ions produced by radiation (3).

Unfortunately it is impossible to put the idea to a direct test because the dielectric properties of protoplasm are so poor that ions which may be produced by radiation cannot be expected to alter its conductivity to a detectable degree. When changes in the conductivity of cells or tissues do occur under the influence of radiation—as has been demonstrated to be the case with blood corpuscles—they are to be attributed to structural changes in the protoplasm as a whole, rather than to the ionization of its molecules (4).

A considerable body of evidence indicates that the effects of α-, β-, γ-rays of radium and x-rays are not only qualitatively alike but vary quantitatively with their ability to ionize air (5), (6). In particular it may be pointed out that the physiological action of groups of β-rays of different velocity is directly proportional to their ionizing power (7). This evidence is quite compatible with the ionization hypothesis and must follow as a result of such a mechanism of physiological action. On the other hand, it is not crucial evidence in its favor, for it must be considered that the physiological effect and the ionization of air are both dependent on the tendency of the various rays to be absorbed by matter, and their parallelism need not indicate any causal relationship between the two.

In the present paper we propose to bring forward evidence of a different sort which indicates that certain characteristics of the reaction of living cells to β-radiation may be predicted quantitatively from a simple theory having as its basis the assumption that ionization is the initial step in the reaction.[1] The experiments have been carried out at Woods Hole using the eggs of Nereis limbata. The reaction of these eggs to radiation and the technique employed in measuring its extent has been fully described elsewhere (5), (8).

When exposed to a uniform intensity of β-radiation the course of the process effecting jelly secretion and membrane formation in the Nereis egg is described by the equation[2]

$$k = \frac{1}{t} \log_e \frac{a}{a - x} \tag{1}$$

in which a measures the total amount of jelly substance in the egg and x the amount changed in the time, t (5). The velocity coefficient, k, for any given lot of eggs is a function of the conditions of radiation, including in particular the intensity. When the total quantity of radiation is the same the change which is produced is greater if low intensities act for a long time, than if high intensities act for a short time (8). From this it may be inferred that the value of the velocity coefficient, k, does not increase in direct proportion to the intensity of radiation but more slowly, so that the radiation becomes less efficient at high intensities.

These considerations have suggested a theory which assumes that some constituent of protoplasm is ionized at a constant rate under any given intensity of β-radiation.[3] The ions so formed may have one of two fates, either they may recombine, or they may lose their ability to do so by entering into combination with some other constituent of the system. The number of ions having the latter fate in unit time determines the rate at which the protoplasmic change finally observed takes place; on it depends the value of the velocity coefficient, k.

If n is the number of ions present at any time, the rate at which ions disappear as the result of recombination will be expressed by αn^2 where α is a constant, the coefficient of recombination. This relation has been

[1] Radium emanation has been placed at our disposal freely through the courtesy of Dr. Charles P. Greenough, Director of the Huntington Memorial Hospital, and of Dr. William Duane.

[2] We propose to employ natural logarithms instead of common logarithms, as in our previous paper (5), in these and subsequent calculations of the values of k. In preparing a paper to follow shortly it has been necessary to do so, and for the sake of uniformity it seems best to follow this practice throughout the series.

[3] We wish to acknowledge our indebtedness to Mr. C. G. Darwin for the development of a simple mathematical treatment of the theory.

demonstrated by Jaffé and van der Bijl to hold for the recombination of ions formed by radium radiations in liquid dielectrics as well as being deducible on theoretical grounds (9).

The rate at which ions disappear from the system by the other process is assumed to be proportional to the number of ions present, and may be expressed by βn, where β is a constant determining the rate of this process. The total rate at which ions disappear will consequently be given by $\alpha n^2 + \beta n$.

Under a constant intensity of radiation an equilibrium will occur in which the rate at which ions are formed will equal the rate at which they disappear as the combined result of these two processes. If θ is a constant depending on the number of ions formed in unit time by one millicurie centimeter of β-radiation, the rate at which ions are formed will be θI, and we may write

$$\alpha n^2 + \beta n = \theta I \tag{2}$$

The value of the velocity coefficient, k, in equation (1) which describes the course of the reaction occurring under any constant intensity depends on the number of ions disappearing from the system in unit time by the process which is determined by βn. Consequently

$$k = \beta n \tag{3}$$

Substituting (3) in (2) we obtain

$$\frac{\alpha}{\beta^2} k^2 + k = \theta \mathrm{I} \tag{4}$$

which relates the velocity coefficient with the intensity of β-radiation under which the reaction occurs. This equation has been put to the test of experiment.

Series of eggs of Nereis limbata were exposed for definite periods of time to β-rays from radium emanation enclosed in slender glass tubes approximately 1 cm. in length. The walls of the tubes were sufficiently thick to stop the α-radiation. The γ-radiation from such tubes is negligible when its effects are compared with that of the β-radiation (10). The intensities were varied by altering the distance between the source and the eggs, and were estimated from the formula of Wood and Prime as described in a former paper (8). The values of k were determined in accordance with equation (1), from measurements of x, the increase in the volume of the perivitelline space, for each intensity, and a the maximal value obtainable for this volume by prolonged exposure. The results of such an experiment are recorded in table 1. The values of k determined experimentally and shown in the third column may be compared with the values calculated from equation (4) on the assumption that

$\theta = 0.003$ and $\frac{\alpha}{\beta^2} = 34$. The agreement is as close as the accuracy of the method would lead one to expect and the deviations vary in sign at random in the course of an eighteen-fold increase in intensity.

On the basis of this and a number of other similar experiments it may be concluded that the theory is compatible with the experimental facts.

The effect of temperature on the relation between intensity of radiation and the velocity coefficient of the reaction of Nereis eggs to β-radiation should enable the general theory underlying the equation $\frac{\alpha}{\beta^2}k^2 + k = \theta I$

TABLE 1

July 18, 1919—Nereis limbata eggs exposed to 39.04 millicuries radium emanation enclosed in glass tube 1 cm. long at various distances. Time of exposure, t, 15 minutes; 25 eggs in each lot. Measured 60 minutes after fertilization.

a(observed) $= 18.2 \times 10^5 \mu^2$
$\theta = 0.003$
$\frac{\alpha}{\beta^2} = 34.0$

I	x	k	k	DIFFERENCE
INTENSITY OF RADIATION	VOLUME PERIVITILLINE SPACE	OBSERVED	CALCULATED	
mc.cm.	$10^5\mu^3$			*per cent*
183.0	14.8	0.1117	0.1132	−1.3
125.0	13.6	0.0920	0.0913	+0.8
70.3	11.3	0.0646	0.0654	−1.2
44.5	9.65	0.0505	0.0496	+2.2
25.8	8.00	0.0385	0.0352	+9.4
16.8	5.95	0.0264	0.0265	−0.4
9.75	4.20	0.0175	0.0188	−7.0

to be put to a further test. In this equation two of the terms, θ and $\frac{\alpha}{\beta^2}$ are constants which are evaluated from experimental determinations of k and I. It is much easier to fit a set of observations to a theoretical curve if there are two arbitrarily chosen constants underlying the position of the curve than if there is only one. It is in accordance with our knowledge of ionization processes to assume that the value of θ, measuring the number of ions formed in unit time by unit quantity of radiation, will be unchanged by an alteration in the temperature of the radiated matter. The effect of temperature must express itself completely through variation in the value of $\frac{\alpha}{\beta^2}$.

In order to test this point an experiment has been carried out in which Nereis limbata eggs were exposed at two different temperatures to various intensities of β-radiation and the corresponding values of k determined. The result is illustrated in figure 1 in which theoretical curves are drawn

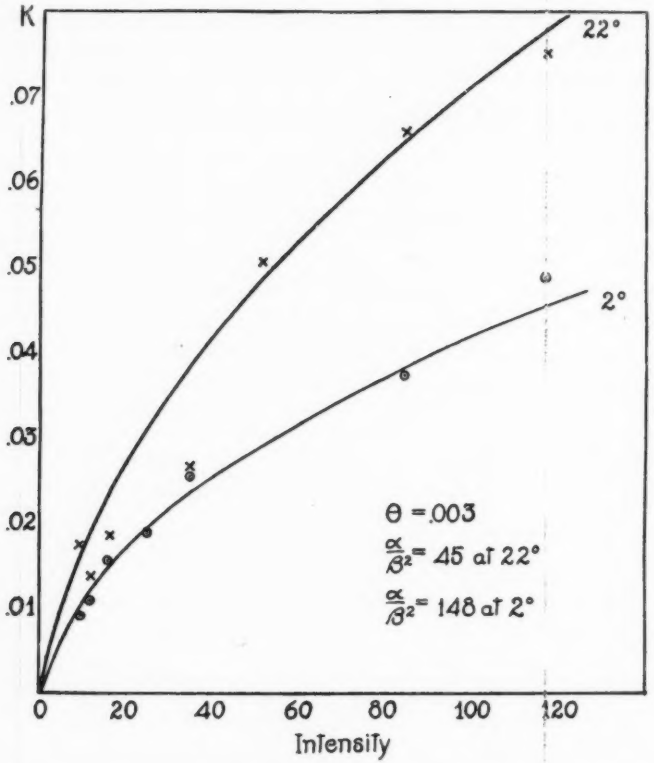

Fig. 1. Curves showing the effect of temperature upon the relation between the intensity of β-radiation and the velocity coefficient of the process effecting jelly secretion in Nereis eggs. Intensities measured in millicurie centimeters along the abscissa, k measured along the ordinate. Experimental points determined at temperatures of 2°C. and 22°C. are based on measurements of 25 eggs exposed for 15 minutes. July 25, 1919.

through the experimental points taking θ to equal 0.003 *at both temperatures* and $\frac{\alpha}{\beta^2}$ to equal 148 at 2°C. and 45 at 22°C. The agreement between the experimental points and the theoretical values is not as close as in the experiment recorded in table 1, for the reason that it is extremely

difficult to control the condition of the eggs long enough to carry out such an extended series of measurements. It is sufficiently good, however, to warrant the conclusion that in terms of the theory the effect of temperature *may* be attributed to a change in the rates of the processes underlying the value of $\frac{\alpha}{\beta^2}$. In this sense the theory is compatible with experi-

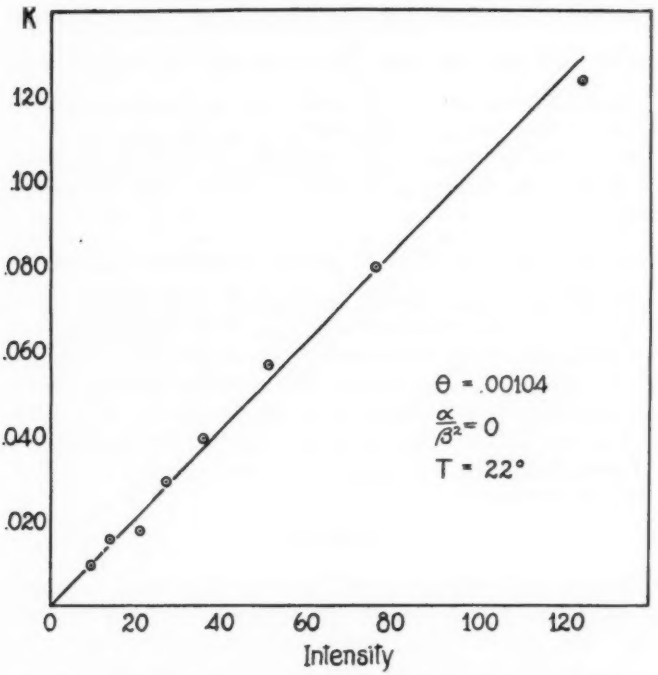

Fig. 2. Curve illustrating a case where the relation between intensity of β-radiation and the velocity of the resulting process effecting jelly secretion in Nereis eggs follows the Bunsen-Roscoe Law. Intensities measured in millicurie centimeters along the abscissa, k measured along the ordinate. Each point based on 25 eggs. July 22, 1919.

mental findings. It must be understood that the experimental results are not sufficiently precise to indicate that θ does not vary at all as the result of temperature change; both constants may be altered somewhat without serious detriment to the "fit" of the curves.[4]

[4] If the present theoretical considerations should prove to be correct and temperature be found to effect the constants in the manner assumed in drawing the

An interesting consequence of the foregoing theory is that under suitable conditions, the response of protoplasm to β-radiation may be expected to follow approximately the Bunsen-Roscoe Law of photo-chemical action. This law states that equal photo-chemical effects are produced whenever the product of the intensity and time of illumination are the same. Since the velocity of a process is inversely proportional to the time required to produce a given effect it follows that the velocity is directly proportional to the intensity of the light. If the conditions of radiation be such that the term $\frac{\alpha}{\beta^2}k^2$ in equation (4) becomes negligible, then the expression becomes $k = \theta I$, which is an implication of the Bunsen-Roscoe Law. This law is consequently a special case of our theory which must hold whenever the conditions are such that the recombination of ions fails to occur or to vary in degree with the intensity of radiation.

We have seen that the value of $\frac{\alpha}{\beta^2}$ may be expected to diminish with rising temperature. Consequently the response of the Nereis eggs should tend to accord with the Bunsen-Roscoe Law if the β-radiation is carried out at a sufficiently high temperature. In certain lots of eggs, this is found to be the case at temperatures within normal physiological limits. Figure 2 illustrates data obtained at a temperature of 22°C. Through the experimental points a line is drawn representing the requirements of the Bunsen-Roscoe Law as expressed by the equation $k = \theta I$, θ being taken equal to 0.00104.

SUMMARY

1. A theory is developed which assumes that some constituent of protoplasm is ionized at a constant rate proportional to the intensity of β-radiation. The ions so formed may either recombine or lose their ability to do so by entering into combination with some other constituent. The number having the latter fate determines the value of the velocity coefficient of the reaction. Mathematical treatment yields an equation which describes accurately the results of experiments upon Nereis eggs.

curves in figure 1, it will follow that the temperature coefficient of k will increase as the intensity increases. This is shown by the following values of Q_{10}, estimated by the equation of Kanitz (11) from values of k given by the theoretical curves in the figure at various intensities.

Intensity	Q_{10}
10	1.27
20	1.28
50	1.30
120	1.31

2. Experiment supports the theory in indicating that temperature may influence the velocity of the process by altering the number of ions which recombine without contributing to the physiological change.

3. The Bunsen-Roscoe Law may be regarded as a special case of the theory. Certain lots of eggs conform to it in their response to β-radiation.

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THE PHYSIOLOGICAL ACTION OF IONIZING RADIATIONS

II. In the Path of the α-Particle

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Received for publication December 6, 1923

We have pointed out recently that such differences as occur in the action of α-, β- and γ-rays and ultra violet light upon the egg of Nereis limbata may be attributed to differences in the absorption of these radiations by protoplasm (1). The fundamental similarity in the physiological phenomena induced by these various radiations suggests strongly that the mechanism underlying their action may in all cases be the same. When a search is made for some physical action common to all these types of radiation it appears that, with the exception of the β-ray, each has the property of causing electrons to become detached from the matter through which it passes and to fly off with a quantity of energy which depends upon the quality of the primary radiation in question. The α-ray liberates the so-called δ-rays, the γ- and x-rays set up secondary corpuscular radiations, while light expels the photo-electrons which account for photo-electric effects. It can scarcely be doubted that such secondary radiations are set up in living tissue by the radium radiations and x-rays for these phenomena depend upon the atomic nature of the absorbent rather than the molecular arrangement of the atoms, which is the sense in which the structure of protoplasm is specific.

The β-ray is, itself, such an electron differing from the various secondary corpuscular radiations only in its greater velocity. If these secondary radiations are important physiologically the mechanism of their action must be essentially the same as that of the β-rays. In the preceding paper of this series (2) we have considered certain evidence which indicates that the effect of the β-ray may be related to its ionizing power. In the experiments described in the present paper we have examined the intensity and the extent of physiological action along and about the path of the α-particle in the hope of testing the inference that the effect of the α-particle is due to its ionizing power and may be related to its ability to set up secondary δ-rays.

If the physiological effects of α-rays are due to ionization set up either directly by the passage of these corpuscles through the protoplasm, or by

δ-rays generated along their paths, it should be possible to show that the physiological effect of the α-ray and its ionzing power vary in a similar way when the velocity changes along the path of the ray. The velocity, V, of an α-particle at any distance, x, from its source is given by the expression $V^3 = a(R - x)$ where a is a constant and R is the maximum range of the particle. It follows that the velocity falls off at a constantly accelerated rate. The change in the energy dE of the α-particle in the distance dx after traversing a distance x is given by

$$\frac{dE}{dx} = \frac{K}{(R-x)^{\frac{1}{3}}} = \frac{K_1}{V}$$

On the assumption that the ionization produced in dx is proportional to $\frac{dE}{dx}$ it follows that the ionization per unit length of path varies inversely as the velocity and will be produced with a constantly accelerated rate as the end of its path is approached. The greater part of the ions are formed close to the end of the path (3).

In practice it is difficult to obtain an intense source of α-particles which produces ionization effects which conform to the foregoing theory with exactness, for while every α-particle derived from a given radioactive disintegration is emitted with the same velocity, small variations in the quantity of matter through which the particles must pass in escaping from the source alter the effective ranges and velocities of the various rays, whose combined effects are measured. The ionization produced in air at various distances from a thin layer of radium has been measured by Bragg and Kleeman (4) and is shown in figure 1A. The essential feature of the curve is that, as the distance increases, the ionization effect falls off, but passes through a distinct series of maxima corresponding to the mean range of the α-particles from each step in the radioactive disintegration.

In our experiments Nereis limbata eggs have been exposed to α-rays from radium emanation confined in a very thin-walled glass bulb.[1] The α-particles from such a preparation are those emitted by the disintegration of radium emanation, radium A and radium C, which have ranges of 4.16, 4.75 and 6.94 cm. in air respectively. The rays escaping from the bulb are, however, far from homogeneous, for not only will the thickness of the glass wall vary in different parts, but different rays will pass through it more or less obliquely. For this reason the physiological effects produced by α-rays from such bulbs usually fail to show any maximal effects at distances corresponding to the end of the range of any group of α-rays. Instead they fall off progressively as does the ionization curve of the α-rays from

[1] Radium emanation has been placed at our disposal freely through the courtesy of Dr. Chas. P. Greenough, Director of the Huntington Memorial Hospital.

thick layers of radioactive matter. We have been fortunate in preparing one bulb with walls thin and uniform enough to emit α-particles sufficiently homogeneous to demonstrate a maximal physiological effect near the end of the range of the particles from radium C and probably also from radium emanation.

In carrying out the experiment eggs were placed in hanging drops at various distances above the bulb of emanation. Since the solid angle subtended by each egg falls off with the square of the distance between the egg and the source, the time, t, of exposure was varied so that $\frac{t}{d^2}$ remained constant. If the rays did not change in their velocity or energy in traversing the distance each egg would have been exposed to an equivalent number and quality of rays and equal effects would have been produced upon it.[2] Any deviation in the results from this equality is due to, and will indicate, changes in the numbers of rays or in their physiological effectiveness as they are absorbed by air. The result is expressed graphically in figure 1B. It indicates that the maximum effect is produced at a distance of about 2 cm. from the center of the bulb and that a second marked maximum occurs at a distance of about 4.7 cm. with a minimum half-way between. Assuming that the mean stopping power of the wall of the bulb is equivalent to 2.15 cm. of air, the end of the paths of the α-particles from radium emanation and radium C would be at 2 cm. and 4.8 cm., respectively, which is in excellent agreement with the result obtained. Inspection of the curve of Bragg and Kleeman shows that the α-particles from radium A cannot be expected to produce a demonstrable deflection of the curve obtained by a method as inaccurate as the physiological one. It also indicates that the minimum effect should appear just mid-way between the two maxima. Since our preparation did not contain the element radium, the portion of their curve corresponding to its effect is of course lacking in ours.

From these considerations we conclude that the physiological effect of α-particles varies with the velocity of the particle in a manner quite similar to the variation of the ionizing power with the velocity. The result is in agreement with the view that ionization is the first step in physiological action. It is not, however, a critical test of this theory, for it goes no further than to imply that the same laws govern the absorption of energy

[2] If the considerations relating to the equivalence of intensity and time shown to hold for β-rays apply to α-rays they would lead one to expect rather greater effects at greater distances. The effect would be a steadily increasing one and would not produce definite maxima. Consequently this consideration does not affect the argument which is developed. An experiment carried out with each lot of eggs radiated for equal periods of time also demonstrated the maximal effect near the end of the range of the α-particles from radium C.

for physiological purposes as govern the absorption of energy for the ionization of air.

The thickness of the affected layer of protoplasm. The foregoing experiment leads to an interesting conclusion with regard to the thickness of the

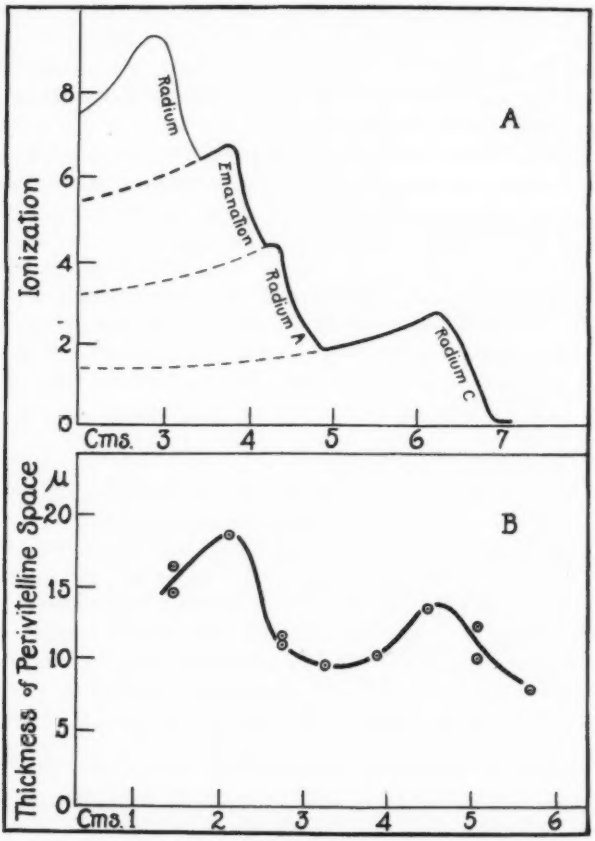

Fig. 1. A. Curve illustrating the intensity of ionization at various distances from a thin layer of radium. After Bragg and Kleeman.

B. Curve illustrating the thickness of perivitelline space of Nereis eggs exposed at various distances to α-rays from radium emanation. The abscissa is displaced to the right to allow for the stopping power of the wall of the bulb and to bring the points of maximal effect into correspondence with those in A.

Experiment of July 14, 1922. 16 mc. emanation in bulb. Time of exposure varied inversely with square of distance—10 min. at 3.3 cm. Fifteen eggs measured for each point.

layer of protoplasm in which the physiological action takes place. Curves such as those of Bragg and Kleeman can only be obtained if the ionization is measured in a thin layer of air—the ionization vessel which they used was actually 0.2 cm. deep. In order to obtain the result shown in figure 1B the layer of protoplasm must also have been very thin, for if a minimal effect is to be produced, as shown at 3.3 cm., it is necessary that the rays from radium C be able to pass through the layer while their velocity is high and their ionizing power low, and enter a portion of the cell where their absorption does not affect the measurements before they give up the greater part of their energy.

Previous results have indicated that the reaction of the Nereis egg with which we are concerned is confined to the alveolar layer of the unfertilized egg which has a thickness of about 4.3 μ. A careful series of measurements of the depth which α-rays penetrate into the eggs, based on the distance between the surface of the egg opposite the side of incidence and the point on the periphery most distant from the side of incidence at which any α-ray effect can be detected, have indicated that for every centimeter decrease in the distance between source and egg the depth of penetration increases 12μ. The alveolar layer would consequently have a stopping power equivalent to $\frac{4.3}{12} = 0.36$ cm. of air. This is a magnitude sufficiently small to give the type of curve obtained.

The volume affected by a single α-particle. It has been shown that the modification of the process of membrane formation and jelly secretion in the egg of Nereis as the result of radiation is due to an alteration of the substance in the perivitelline space which under normal conditions is secreted to form the jelly, and that in consequence this substance cannot escape from the egg (1). If it be assumed that each α-particle alters all the jelly substance within a certain distance from its path it is possible to calculate the volume of the cylinder in which the affected material is concentrated. The rate of change in the quantity of altered material under uniform intensity of β-radiation is given by $\frac{dx}{dt} = k\ (a—x)$ where a is the initial quantity of jelly substance and x the amount changed in time, t. On integration this becomes $k = \frac{1}{t} \log_e \left(\frac{a}{a—x}\right)$. Since the effect produced is identical in nature to that of β-radiation it is safe to assume that the rate of change under α-radiation follows a similar law. In demonstrating this relationship the quantities were deduced from measurements of the volume of the perivitelline space of radiated eggs. We may consequently take a and x to measure the volumes occupied by the jelly substance which these symbols designate. If every α-particle passed through the perivitelline space without falling on the path of a previous particle, the volume altered

by a single particle would be given by dividing x, the volume altered in a given time by the number of α-particles entering the egg in that time. This condition only holds at the initial instant of radiation, when x is infinitesimal, and the rate of change is given by $\frac{dx}{dt} = ka$. The fraction of the total volume altered in unit time is $\frac{ka}{a}$ or k. We have assumed that the affected volume is distributed in a cylinder of uniform cross section throughout the entire depth of the perivitelline space. The fraction of surface equivalent to the sum of the cross sections of all these cylinders affected in unit time is consequently given by k.

The number of α-particles passing through unit surface at a distance, d, from the source in unit time is given by $\frac{QN}{4\pi d^2}$ where Q is the number of millicuries of emanation in the source and N the number of α-particles given off per millicurie which may be expected to traverse the distance, d. The surface equivalent to the cross section of the cylinder affected by one α-particle is

$$\frac{4\pi d^2 k}{QN}$$

In the experiment illustrated in figure 1B, if we consider the lot of eggs at a distance, d, of 3.3 cm. it is probable that only the α-particles from radium C were effective. N was therefore 2.04×10^9 per minute (3). Q was 16.3 millicuries. k, calculated from the experimental data, was 0.058. Applying these values the area of the cross section of the cylinder of action of the α-particle comes out as 2.3×10^{-10} sq. cm.; the radius 8.56×10^{-6} cm. The thickness of the alveolar layer is 4.3×10^{-4} cm.: the volume of the cylinder of action is consequently 9.9×10^{-14} cc. The α-particle is of atomic dimensions, having a radius of the order of 10^{-8} cm. It is clear from the preceding calculation that its effects must extend beyond the space it actually traverses by a distance nearly a thousand times as great as its own radius. The calculation is designed to give the minimum value of the dimensions of the cylinder for it assumes that all the radium C particles extend to 3.3 cm. from the source; it is based on eggs placed at such a distance that the effect of the α-particles is minimal because of their high velocity; and finally it implies that the altered jelly substance is all concentrated in the cylinder, unmixed with unaffected jelly substance. The assumption that all the α-particles from radium C extend for 3.3 cm. is undoubtedly subject to correction, for a certain number of α-particles will pass through the bulb obliquely and will not retain enough energy to carry them on through this thickness of air. Could this error be corrected probably it would not enlarge the dimensions to a

very significant degree. If the calculation is based on the effects produced at a distance of 4.5 cm. where the action of the α-particles from radium C is maximal the area of the cross section of the cylinder comes out 6.2×10^{-16} sq. cm.; its radius 1.4×10^{-5} cm.; its volume 2.7×10^{-13} cc. At this distance it is still more probable that all the particles do not reach the eggs. The difference in the dimensions of the cylinder when computed at different parts of the path makes it appear quite unsafe to assume that all of the material within a certain distance of the actual path of the α-particle is altered and none beyond this distance. If this is not the case, the distance at which action can take place may be much greater than the measurements indicate.

Rutherford (5) has determined the radius of the cylinder of action of the α-particle in destroying the centers which give rise to scintillations in certain minerals when they are bombarded with these radiations. The results are as follows:

Zinc sulphide	1.3×10^{-7} cm.
Willemite	2.5×10^{-7} cm.
Barium platinocyanide	1.6×10^{-5} cm.

The latter result agrees closely with the radius estimated for the Nereis egg.

The magnitude of the radius of the cylinder of action of the α-particle in the case of the Nereis egg may be explained by assuming that in ionizing the protoplasm, electrons are caused to fly off from atoms directly in the path of the α-particle with sufficient energy to travel at least 10^{-6} cm. Such electrons are known to be liberated when α-particles pass through various metals and are known as δ-particles. Their velocity of expulsion is small but is sufficient to enable them to escape from a layer of matter 10 or 20 molecules deep. Bumstead (6) has found that the emission of δ-particles varies in exactly the same way as the ionization along the path of the α-particle. The apparently greater volume affected by the α-particle near the end of its path is concordant with this fact. While it cannot be said that the experimental evidence is a critical test of this explanation, the facts are entirely in accord with it.

It has been suggested from time to time that the physiological effects of α- and β-rays are in some way connected with the electrical charge carried by these particles into the protoplasm. In a recent paper by Zwaardemaker (7) the antagonistic effects which are claimed to exist between certain radioactive elements are explained by the neutralization of the action of the positive charges of the α-rays from one element by the negative charges of the β-rays from the other. That the residual charge imparted by the ray when it comes to rest should be an important factor in producing physiological effects seems highly improbable when it is considered that each ray may be expected to produce a hundred thousand pairs of ions before

it comes to rest, and that each of these ions carries a charge of the magnitude of that of the α- or β-ray. Further it has been found that the change in the process of jelly secretion in the Nereis egg is affected alike not only by the oppositely charged α-rays and β-rays, but by the uncharged x-rays and by ultra-violet light (1). The effect of β-rays of different velocity upon this process has been shown to be proportional to the ionizing power of the rays, and not to the number of rays, or negative charges, entering the cell (8). The present estimations of the volume affected by each α-particle add to the improbability that its action is due to its charge. If the altered material be conceived to be concentrated in a sphere about the α-particle in its position of rest the estimated volume of 9.9×10^{-14} cc. would occupy a sphere of 2.9×10^{-5} cm. radius. Inasmuch as most of the α-particles from radium C would possess sufficient energy after travelling 3.3 cm. to penetrate well beyond the alveolar layer of the egg, only a small proportion of them can be assumed to leave their charge in the region in which the reaction takes place. The volume which must be attributed to the action of each α-particle and the radius of the sphere in which it would lie is much larger than these figures indicate, and it consequently becomes very difficult to conceive of the charge of the α-particle producing an effect at such a distance or on such a mass of matter.

SUMMARY

The physiological effect and the ionizing power of the α-particle vary in a similar way as the velocity of the particle changes along its path. The α-particle exerts an effect at a distance of at least 10^{-5} cm. from its path. This phenomenon is attributed to the action of secondary δ-rays.

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THE SCROTUM AS A TEMPERATURE REGULATOR FOR THE TESTES[1]

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Received for publication December 12, 1923

During the course of the work on sex glands in this laboratory within the last few years, particularly the studies on the testes, we have had forced on our attention the intimate relationship that exists between the scrotum and the testes. Thus in an animal with open inguinal canals (rat, rabbit, guinea pig, etc.) the testes may be periodically drawn into the peritoneal cavity to redescend later into the scrotum; the testes of these animals are normal under such conditions. If, however, the testes are prevented from returning to the scrotum their period of sexual potency is brief indeed. The testes of a guinea pig may be so retained by sewing shut the internal opening of the inguinal canal, by a slight suture of the testis to the peritoneum without closing the canals, or by cutting the very slight mesenterial-like connection of the lower part of the epididymis to the bottom of the scrotal sac, thus allowing the testis to remain free in the peritoneal cavity with the inguinal canals open. Following the latter procedure the testis, in some cases, returns to the scrotum; more often, however, it remains free in the abdomen without adhesions, with its blood vessels, nerve supply and vas deferens intact. Peritoneal retention is rapidly fatal to the generative tissues and in a brief course of time the testis is histologically typical of the naturally occurring cryptorchid or undescended testes such as have been described for the pig, sheep, horse, man and other mammals.

The germinal epithelium of a guinea pig testis is found to be highly disorganized after retention within the peritoneal cavity from five to seven days. Many times the germinal epithelium is so completely degenerate in fifteen to twenty days that the seminiferous tubules have been reduced to a single layer of poorly stained cells occupying a position immediately within the basement membrane. These have usually been considered to be Sertoli cells and not germinal cells in the strict sense, though it appears probable that some of these cells are in reality

[1] This investigation has been aided by a grant from the Committee on Sex Research of the National Research Council; grant administered by F. R. Lillie.

spermatogonia. The intertubular tissue usually becomes decidedly prominent after one month; at three or four months' retention within the abdominal cavity the space between the atrophic seminiferous tubules is packed with interstitial cells (Leydig cells). The testes do not regain functional activity as long as they remain in the peritoneal cavity, but should they return to the scrotum functional activity may be resumed; in animals possessing a scrotal sac the testes may fail to descend, in which case they are always sterile. The limit of degeneration after which recovery is no longer possible has not been determined, but testes in which the epithelium is reduced to a single layer of cells next the basement membrane have been found to reconstitute normal tubules within a few months after being returned to the scrotum by operative procedures.

The testis-scrotal relationship is again emphasized by a study of testis grafts. In brief it may be said that out of more than one hundred testis grafts none have been found that contained spermatozoa excepting those that had been transplanted onto the walls of the scrotal sac (tunica vaginalis) and therefore within the scrotum (for further details of this see (2), (3)). What, then, is the nature of this delicate relationship that exists between the generative part of the testis and the scrotum?

By a process of elimination it appears that the causal factor for degeneration must reside in a differential temperature relationship between the testis and its environment, the environmental temperature being under the control of the scrotum. Crew (4) has advanced the idea that higher temperatures might account for the aspermatic condition of undescended testes. Moore (2), (3) has interpreted the results of experimental cryptorchidism on this basis, there being no other factor that would explain the conditions encountered, and Moore and Oslund (5), (6) have gone a considerable way in giving actual proof that the temperature relationship is the correct explanation.

The scrotum is an outpouching of the skin lined by a thin membrane that has been derived from the peritoneum—the tunica vaginalis; in the rodent, in particular, this connection with the peritoneum has not been severed. The scrotal skin is very thin and well supplied with sweat glands; there is an absence of subcutaneous fat; the muscle layer is extremely thin and the blood vessels of the testis are distributed profusely over its surface. It is well known that the character of the scrotum varies with different temperatures. In high temperatures it relaxes to as full a pendent condition as the size will permit whereas in lower temperatures it is contracted, greatly thickened, and the testes are drawn more closely to the body. Thus on hot days the rat scrotum is relatively huge in size and may protrude considerably in a post-anal position, whereas on cold days it may be so much reduced as to pass unnoticed except on very close examination; indeed the testes on such occasions are more

abdominal than scrotal. We see in such an arrangement the physical possibilities of a regulator of temperature and it is on such an assumption that our procedures have been based.

If the scrotum does possess such important regulatory capacities as controlling the environmental temperature of the testes, and the latter depend upon such a control for their well-being, it would appear that proper covering or external insulation from heat loss would cause the germinal cells to degenerate. This has actually been found to hold for the sheep by Moore and Oslund. By secure, though loose, encapsulation of the scrotum in woolen materials for a period of less than three months, the testes were found to have been so seriously affected that spermatozoa were entirely absent; many seminiferous tubules had lost their germinal epithelium and all were in various stages of degeneration; no spermatozoa were present. The type of degeneration was very similar to that found in testes that had only shortly before been forcibly retained within the peritoneal cavity.

Up to this point in the accumulated evidence of the temperature relationships and degeneration, it had merely been assumed that the actual temperature in the scrotum was less than that in the peritoneal cavity, and that the lower scrotal temperature was due to the regulatory capacities of the latter structure. Investigation has proved this assumption to be a reality. We have measured directly, and at the same moment, the peritoneal temperature and the scrotal temperature and find that the scrotal temperature is consistently, indeed without exception, lower than the peritoneal temperature. An account of these observations follows.

Observations. Our investigations have been limited to animals from which we have derived the gradual chain of evidence that has pointed out the physiological relationship existing between the testes and the scrotum: i.e., the guinea pig, rat and rabbit. In each of these animals the inguinal canals are open and ready access is had from the peritoneal cavity into the cavity of the scrotum.

We have been more concerned with relative than with absolute temperatures and have, therefore, employed only simple procedures. To obtain absolute temperatures would involve the use of more complicated procedures and apparatus such as thermo-couples. In brief, our methods have been as follows: The animal was securely fastened to a table; two small abdominal areas were shaved, sterilized and locally anesthetized; apertures of sufficient size to admit the bulb of a relatively sensitive thermometer were made through the body wall and peritoneum, one slightly above the other in the mid-ventral line. We have avoided a general anesthesia as this is known to interfere with the normal heat regulating capacities of the body. For temperature records we employed two 50°C.

thermometers, graduated to one-tenth of a degree. The temperature readings on each thermometer were taken at one or two minute intervals until they were approximately constant for the peritoneum. At the desired moment the lower thermometer, without removing the bulb from the body cavity, was slanted sufficiently for the bulb to pass through the open inguinal canal alongside the testis into the scrotum (saccus vaginalis). Readings of scrotal temperatures and peritoneal temperatures were continued for a few minutes only. The scrotal thermometer was then redrawn into the peritoneal cavity and readings taken until the two thermometers again registered the peritoneal temperature for a short time. We did not prolong the time unnecessarily in order to register the lowest possible temperature in the scrotum. In any observation the animal was off the table within thirty minutes from the beginning of the experiment. At the end, both thermometers were removed, and with a single stitch the apertures in the body wall were closed. In every case the animal recovered from the experiment without any indication of discomfort. Temperature readings of the scrotal thermometer were not only controlled by peritoneal readings on the same thermometer before and after its period in the scrotum, but the upper thermometer as well recorded a control peritoneal temperature throughout the entire observation.

We very soon appreciated the influence of external room temperatures and we have, therefore, carried out the procedures at different room temperatures varying from 37°C. to 14°C.

It was a surprise to find not only such a marked difference between the peritoneal temperature and the scrotal temperature at the same moment but also to find such a great variation of these differences in the different type of animal used for the observation. The white rat gave the greatest difference between the temperatures of the two localities, the rabbit the least, with the guinea pig taking an intermediate position in this respect. In the rat, observed at an external room temperature of 14°C., a difference of 7.5°C. was found to exist between the peritoneal cavity and the cavity of the scrotum. Table 1 is one set of readings taken as recorded in our notebook; other representative observations for the three different animals are expressed in the form of a curve (see figs. 1 and 2). Here the ordinates represent degrees in Centigrade and the abscissae the time of the experiment in minutes; as constructed the curves represent the relative temperatures within the two cavities at the same moment.

It should be emphasized that no exceptions to the above conditions have been found; in each animal observed the scrotal temperature was found to be lower than that of the peritoneal cavity. So far we have not made observations on animals in which the scrotal cavity (saccus vaginalis) was separate from the peritoneal cavity—i.e., with the inguinal

canals closed. One would suppose, however, that insofar as the two cavities would be more completely removed from each other, the temperature difference would be even greater than where the open connections exist between them.

DISCUSSION. We believe the observations recorded above complete the chain of evidence, many links of which have only recently been added, that points very definitely to the function of the scrotum as a local regulator of temperature for the testis. Within the peritoneal cavity where the temperature is higher than their normal environmental temperature, the testes rapidly degenerate and do not regain a normal condition as long as they are so situated; if, however, they are returned to the scrotum

TABLE 1

Young adult guinea pig

TIME	DEGREES CENTIGRADE	
	Lower thermometer	Upper thermometer
5:03 p.m.	35.9	35.81
5:05	36.85	36.15
5:07	37.1	37.09
5:09	37.5	37.3
5:11	37.6	37.4
5:13	37.5	38.1
5:15	37.9	37.9
Lower thermometer inserted into scrotum		
5:17	36.85	38.21
5:19	36.1	38.3
5:21	36.1	38.3
Lower thermometer redrawn into peritoneal cavity		
5:23	37.85	38.35
5:25	38.05	38.51
5:27	38.3	38.53
Room temperature	30.85	30.9

even after having lost a vast majority of the generative tissue, they will recover and again produce normal germ cells. So far only scrotal grafts of mammalian testis have proven capable of differentiating spermatozoa. A peculiar testis-scrotal relationship seems necessary for complete germ cell differentiation.

On the other hand, a scrotum insulated externally against loss of heat leads to testicular degeneration. No other explanation for this fact is apparent than on the basis of an interference in the thermo-regulatory function of the scrotum. Furthermore, the proof that higher temperatures are fatal to the generative tissues is given from actual observations on the effect of the local application of higher temperatures to the scrotum (7), (8). Heat applied to the scrotum externally *in one application* pro-

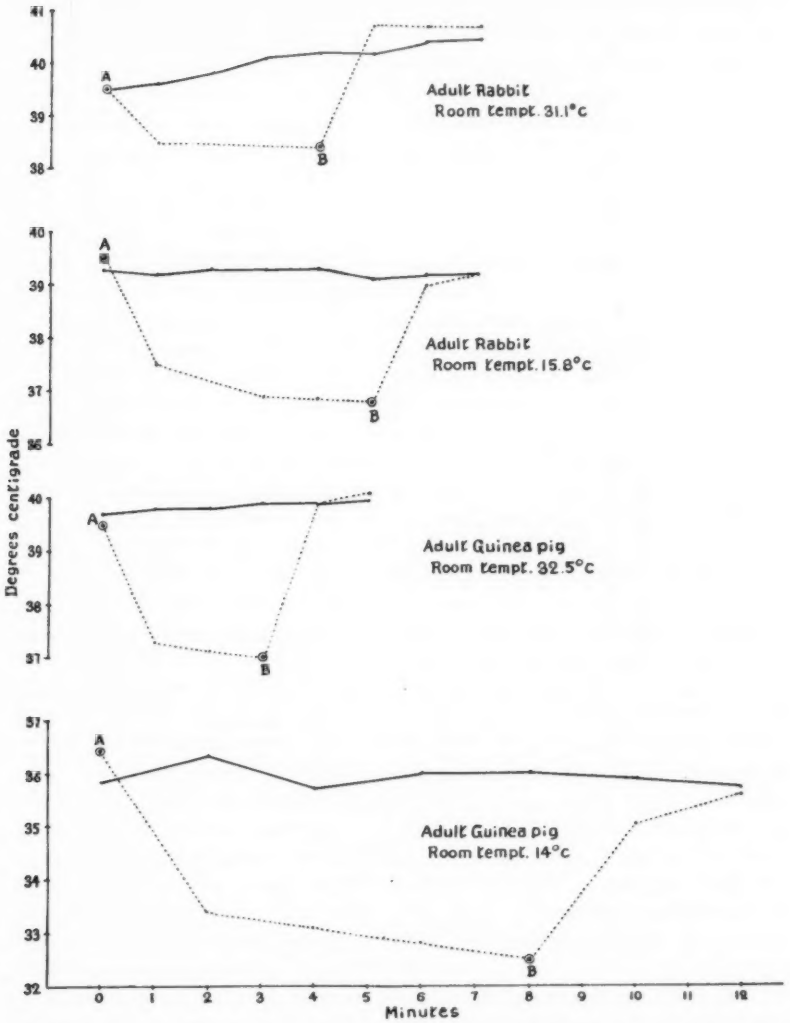

Fig. 1. Graphs showing temperature of peritoneal cavity in comparison with temperature of scrotum in the rabbit and guinea pig, at different room temperatures. In each case (fig. 2 also) the peritoneal cavity temperature (*upper thermometer*) is indicated by a solid line. At point *A*, lower thermometer pushed into scrotum alongside testis; decline in temperature indicated by dotted line. At point *B*, lower thermometer retracted into peritoneal cavity; temperature increase follows.

duces partial or complete tubular degeneration. Finally, we have shown that the actual normal scrotal temperature is appreciably lower than the

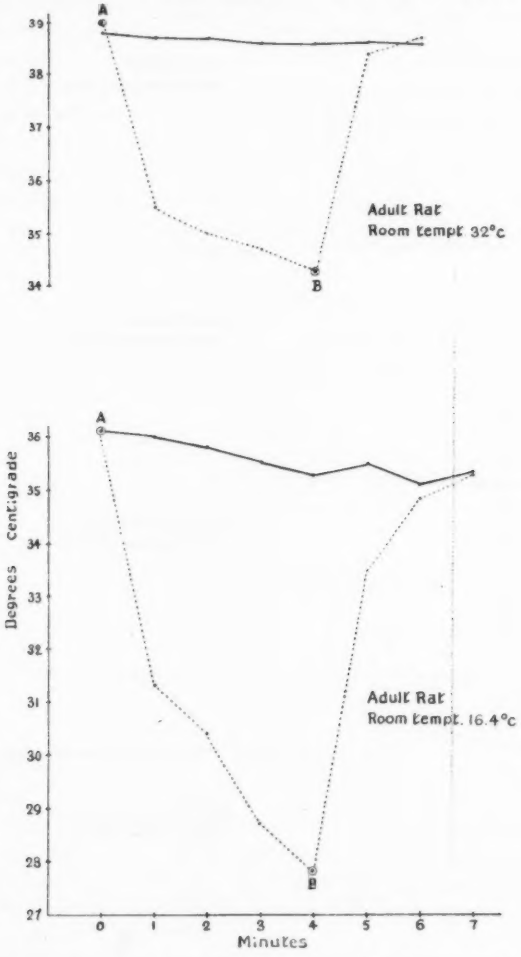

Fig. 2. Graphs showing peritoneal cavity temperature in comparison with scrotal temperature in the white rat, at different room temperatures. Procedures and temperature changes indicated as in figure 1.

peritoneal temperature, and that the difference becomes greater as the external temperature falls. In nature this is compensated for by scrotal contraction, thus drawing the testes nearer the body of the animal; a

warmer environment leads to the reverse adjustment and the scrotum becomes more pendent, thinner, and farther removed from the body.

We thus have an explanation on a simple thermo-regulator basis for such conditions of the testes as are found in cryptorchidism (normal or experimental) or the effects that have been reported for vasectomy (9), and for many other conditions causing testicular degeneration. We do not, however, claim to account for all tubular degeneration on this basis for there still remain those conditions such as x-ray treatment, vitamine deficiencies, etc., concerning which evidence is lacking as to temperature phenomena.

We believe also that the evidence is sufficient to assign to the scrotum the function of a temperature regulator for the testes. Cunningham (10, p. 147) has said "Various causes have been suggested for the formation of the scrotum, but no one has ever been able to suggest a use for it." Now that a function has been suggested, if indeed not actually demonstrated for it, in those animals equipped with such a structure, it should be recalled that various graded conditions are found even in its production. The necessity for such a control of temperature must be limited to the mammalian group, as these alone possess a scrotum. Is it not intimately correlated or perhaps indispensably bound up with the entire evolution of the mammalian group?

Several members of the group are able to exist without such a mechanism. The monotremata show no approach to such a condition. Here the reptilian position of the testis is retained; the testes are located in the vicinity of the kidneys. In many forms there is definite indication that the descent of the testis has been a gradual process. In some it has descended into the pelvis and in fact may lie in a small depression—not a real pendent projection—on the anterior abdominal wall (bats, sloth, etc.). Practically all the aquatic mammals lack a scrotum and even such members as the elephant, hyrax and others have not found such a definite structure necessary. A further step in its development is seen in rodents wherein the scrotum is but an outpouching of the peritoneum covered by a thin layer of skin, an open connection with the peritoneal cavity being retained. The final mammalian condition is a complete separation of the saccus vaginalis from peritoneum by closure of the inguinal canals.

In the lower mammals the need for or, if it be so preferred, the adaptation to, the thermo-regulatory capacity of the scrotum, appears either not to exist, or to be compensated for by an inherent difference, perhaps related to actual temperature variations.

The various stages in the development of a definite scrotum may well be associated with the position of the testes in relation to the anterior abdominal wall. Some animals have a somewhat less thick skin of the

body wall where the testis rests against it and it is possible that adjustment to temperature may be made in this way. We have observed that a thermometer in the peritoneal cavity with the bulb against the anterior abdominal wall registers an appreciably lower temperature than another one whose bulb is buried in the viscera. A comparison of all mammalian body temperatures, and the extremes of its variation, correlated with the testis and scrotal condition would be extremely interesting. Since we found an appreciably lower peritoneal temperature in contact with the anterior abdominal wall of a rat, there are good grounds for believing that it would be possible to so graft a testis underneath the skin, i.e., outside of the scrotum, that germ cell differentiation might be complete. In subcutaneous grafts of guinea pig testes seminiferous tubules have been found with active spermatogenesis in progress and an epithelium almost normal (see fig. 2, Moore (11) p. 380), but to date no subcutaneous grafts have been observed to contain spermatozoa differentiated in the transplant.

It appears somewhat idle to speculate upon the inherent causes of the production of such a structure as the scrotum, and it is not our particular desire to attempt to account for it upon any specific evolutionary hypothesis. Cunningham (10) has devoted considerable discussion to an explanation of its evolution on a Lamarkian basis. However, the apparent parallelism existing between its gradual acquisition and that of the rise of the female mammalian condition, placental gestation, appears somewhat striking and one could well suppose that the acceptance of an explanation of one condition would also serve for the other. In each newly developed characteristic—scrotal origin, and placental gestation—the change has been a gradual one from reptilian to higher mammalian types, with distinct steps easily recognized. Neither condition is indicated in the lowest mammal group, the monotremata. Marsupials present an approach to placentation, and higher groups show graded stages in the perfection of the condition. The birds, in respect to both testicular descent and placenta, are to be excluded entirely from consideration; obviously the testes in this group are not so heat-susceptible as the testes of mammals.

Whatever the conditions may have been that necessitated the origin and development of such a thermo-regulator condition for the testes as appears without question to exist, or whatever its relationship to the evolution of the mammalian group may have been, we are made aware of an extremely interesting, and beautifully correlated mechanism that is not without some practical application from the eugenics point of view.

SUMMARY AND CONCLUSIONS

1. We have demonstrated that the temperature on the inside of the scrotum is decidedly lower than that of the peritoneal cavity at the same moment.

2. The difference in the temperature of the two cavities varies as the external environmental temperature varies.

3. A needed link in the chain of evidence that seminiferous tubule degeneration in cryptorchid testes, natural or experimental, is due to the effects of an abnormally high peritoneal cavity temperature as compared with that of the scrotum or the normal environmental temperature for the testes, has been added.

4. We believe the evidence presented is sufficient to warrant the conclusion that physiologically the scrotum is a local thermo-regulator for the testes, and inferentially we see the possibilities of its having contributed materially to the evolution of the mammalian group.

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EXPERIMENTAL DEHYDRATION:

Changes in Blood Composition and Body Temperature

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Received for publication December 12, 1923

Lack of sufficient water in the body is a serious condition in certain diseases. It may result from insufficient intake or excessive excretion. Both tests have been made experimentally on man and animals, and definite circulatory changes have resulted. McGee (5) has graphically described the effect of the extreme heat of the desert on man when water is not available. Several observers who have studied the effect of water abstinence on animals have repeatedly noted changes in the blood, besides the loss of weight. Excessive excretion of water from the bowel as it occurs in severe types of diarrhea causes marked dehydration. In cases of Asiatic cholera and acute diarrhea of infants, the dehydration may be sufficient to cause profound circulatory changes and death. Balcar, Sansum and Woodyatt (1) have emphasized recently the effect of rapid loss of water on the temperature-regulating mechanism of the body. They demonstrated experimentally that this rapid loss by diuresis following intravenous injection of various hypertonic solutions may cause a rise in body temperature. Certain experiments that I made early in 1916 led me to believe that strong hypertonic solutions of saccharose, when injected intravenously, caused marked diuresis and circulatory changes without a distinct rise in body temperature. This finding suggested a method which I have employed in the following experiments for studying in detail the changes that might occur in a dehydrated animal. The saccharose and glucose solutions have proved equally satisfactory. The advantage of this method is the production of severe dehydration, without an infectious etiologic factor.

Experimental procedure. The experiments were carried out entirely on female dogs. Their urine was free from albumin, and they presented no evidence of distemper. No food or water was given on the day of the experiment; in a few instances no food and only a small amount of water was allowed during the previous twenty-four hours. The animals were given, intravenously, a 50 per cent solution of either saccharose or glucose, the usual amount injected being 8 grams of saccharose and 9 grams of glucose for each kilogram of body weight each hour for two hours. The best

quality of commerical saccharose (highest grade of white granulated cane sugar) Merck's highest purity glucose, Pfanstiehl's special glucose, and the glucose issued by the Bureau of Standards, Washington, were used. In the earlier experiments the solutions were sterilized by boiling, but after the detection of certain toxic phenomena, the weighed amounts of sugar were dissolved in sterile boiling water. The injection was given continuously by means of the Woodyatt automatic pump (14). No anesthetic was required. The urine was collected accurately by the use of a retention catheter, so that the water lost by diuresis was measured quantitatively. The body temperature was taken by rectum every half-hour. The animal was weighed before, and at stated intervals during the experiment. Initial control estimations were made of certain blood constituents.[1] There was marked dehydration under the foregoing conditions shortly after the injection was finished, but in order that the circulation might reach a state of equilibrium, the animal was left on the table and observed closely for the next three to five hours. At this time the water loss and decrease in body weight, as well as the circulatory changes were determined. The animal was then placed in a metabolism cage and permitted to drink measured quantities of water for the next twenty-four hours. In certain experiments it was not possible to measure the water ingested during this period, as vomiting sometimes occurred. In order to obviate this chance of error, a few dehydrated dogs were given 0.5 per cent sodium chlorid solution intravenously in amounts equal to the water lost. The injection was given slowly over a period of two hours. At the end of twenty-four hours, when the water was taken by mouth, or the two-hour period of sodium chlorid injection, the blood was again studied. Table 1 contains detailed data of a typical experiment.[2]

Toxic phenomena with boiled solutions. During the earlier experiments with saccharose, three animals died within a half-hour to twenty-six hours after the completion of the injection. Muscular twitchings were marked and persistent, the dogs became very weak, the rectal temperature rose and circulatory failure rapidly developed. Necropsy revealed definite enteritis in two animals; in the other, there were slight endocardial hemorrhages, and questionable acute nephritis. These lesions did not appear sufficient to cause death. As one animal had been given a larger dose of saccharose than usual, 8.8 grams for each kilogram of body weight, each hour, death was attributed to too rapid dehydration, although the actual water lost by diuresis was no greater than in the successful experiment. Similar results were observed in three dogs given glucose injections. The usual dose

[1] These specimens of blood were obtained by venous and arterial puncture with needle and syringe. Control blood and plasma volume determinations were always made several days before the injection.

[2] It was not possible to give complete data of the eighteen experiments.

was given to two animals, while the amount was reduced to 8 grams for each kilogram for each hour for the third. Muscular twitchings were marked, the rectal temperature rose, and death resulted within a similar period of one-half hour to twenty-six hours. At necropsy, mild enteritis was found in two animals (table 2).

TABLE I

Dog G173. Saccharose, 8 grams for each kgm., injected hourly for two hours

DATE	HOUR	WEIGHT, KGM.	CHANGE IN WEIGHT, KGM.	TEMPERATURE, 0°C.	BLOOD		URINE, VOLUME CC.	REMARKS
					Hemoglobin, per cent	Whole blood viscosity		
3-19-23	9:30 a.m.	11.4		38.8	120	4.5		
	9:40 a.m.							Injection begun
	10:10 a.m.			38.2			85	
	10:40 a.m.			38.5			225	
	11:10 a.m.			38.4			365	
	11:40 a.m.			38.5			370	Injection finished, 365 cc. 50 per cent saccharose
	12:00 m.	10.34	−1.06	38.8	139	7.9		Total urine for two hours 1045 cc.
	12:10 p.m.						140	
	12:40 p.m.			39.0			65	Retching
	1:10 p.m.			38.7			45	
	1:40 p.m.			38.4			30	
	2:10 p.m.			38.3			20	
	2:40 p.m.			38.1			25	
	3:10 p.m.			38.0			5	
	3:40 p.m.			38.3	166	11.0	9	Total water lost in urine 1019 cc. (89 cc. for each kgm. of body weight)
	4:00 p.m.	10.11	−1.29	38.0				Injection of 0.5 per cent sodium chlorid begun
	4:40 p.m.			37.4			10	
	5:00 p.m.			37.3	135	5.7	5	
	6:00 p.m.	11.1	+0.99	37.7	111	4.1	40	Injection of 1118 cc. of 0.5 per cent sodium chlorid finished. No stools during experiment. Temperature rise 38.8 to 39.0

The severe general toxic condition of the six animals, and the fact that eighteen were not thus affected by the experiment, naturally indicated some added common etiologic factor. It is quite possible that the original water reserve of these animals was so reduced that the tissue could not endure the extreme and rapid dehydration. It is also possible that the hypertonic

sugar solutions, under certain conditions, contain toxic substances. As the solutions had been sterilized by boiling for a few minutes, this procedure was omitted in subsequent experiments, the saccharose and glucose being dissolved in boiling water, and toxic phenomena did not occur in ten consecutive experiments.

The animals were observed closely for the development of abnormal clinical signs or symptoms. The systolic blood pressure was noted in the femoral artery, during the dehydration period, 0.5 per cent novocain being

TABLE 2

Six experiments with boiled solutions

DOG	WEIGHT, KGM.	AMOUNT INJECTED, GM. FOR EACH KGM. EACH HOUR	LOSS OF BODY WEIGHT		LOSS OF URINE		TEMPERATURE RISE 0°C. RECTAL	TIME OF DEATH AFTER INJECTION, HOURS	NECROPSY FINDINGS
			Kgm.	Per cent	Body weight, per cent.	Cc. for each kgm.			
Saccharose									
B.....	9.4	8.8			7.6	76	38.5 to 42+	0.5	Gastroenteritis, pulmonary edema
F319.....	10.1	8.0	1.0	9.9	8.8	88	38.7 to 41.5	1.0	Diphtheric enteritis
F293 (2)..	16.1	8.0	2.45	15.2	10.3	103	39.3 to 41.5	26.0	Subendothelial petechiae in the left ventricle and auricle. Acute nephritis?
Average.....				12.5	8.9	89	Maximal 4°+		
Glucose									
B and W.....	7.8	9.0	0.6	7.7	6.5	65.7	40.5 to 41.5	0.75	Negative
G79.....	8.2	8.0	0.8	9.75	9.0	90	39.3 to 42.3	0.5	Mild enteritis
G52.....	10.2	9.0	1.0	9.8	8.3	83	39.0 to 40.3	26.0	Gastroenteritis
Average.....				9.1	7.9	79	Maximal 3°		

used as a local anesthetic. The rectal temperature was determined by means of a standard centrigrade clinical thermometer. Estimations in the venous blood consisted of *a*, the hemoglobin content by the Haldane-Palmer method (8); *b*, hematocrit, 6 to 10 cc. of oxalated blood in 10 cc.

graduated centrifuge tubes;[3] *c*, whole blood, plasma and serum viscosity with the viscosimeter of Hess (3); *d*, whole blood and plasma volumes by the dye method of Keith, Rowntree and Geraghty (4); and *e*, plasma chlorides according to Rappleye and Smith (9), (11). Estimation of the oxygen content of venous and arterial blood was made by means of the Van Slyke (13) gas pipet. The viscosity of injected hypertonic sugar solution was also determined in the viscosimeter. It was found to vary between 5.8 and 7.4 times that of distilled water.

RESULTS: *Clinical observations.* During the two hours the injection was made, the dog usually remained quiet and except for occasional retching, appeared comfortable. In the subsequent period of dehydration it seemed weak and apathetic, although able to stand and run about. Occasionally there were short periods of shivering and of panting, with deep respiration, general restlessness, even muscular twitchings, and the passage of stools. These symptoms, when present, occurred shortly after the injection was finished and were of short duration. One of the animals did not have any symptoms, while several retched only. The animals were thirsty, for when allow to run about they always went directly to any receptacle that appeared to contain water. The majority of the animals, during the period of dehydration, occasionally passed mucus with small amounts of blood, by rectum. At the end of from three to five hours, after discontinuing the injection, the feet were cold and often there was visible cyanosis in the mucous membrane of the mouth, tongue and in the skin over the abdomen. Dogs that were given sodium chloride solution intravenously, equivalent to the amount of water lost during diuresis, were restored rapidly and often strikingly. The cyanosis disappeared, the extremities became warm, and the animals ran about eagerly searching for water. Eighteen dogs submitted to this experiment, recovered promptly, and within a few days they appeared lively and normal in every way. The procedure was successfully repeated on one animal, one month after the first experiment. It has remained apparently normal for several months.

Changes in weight, body temperature and excretion of urine. Diuresis began promptly after the beginning of the injection. The output of urine increased rapidly, reaching a maximum in from one to one and one-half hours. Excretion continued at this rate for one-half to one hour, and in a given one-half hour period, amounted to as much as 17 cc. a minute. The rate of secretion of urine was more rapid with the injection of glucose than with saccharose. Soon after the injection of glucose was finished, the excretion of urine began to diminish and ceased at the end of two hours, while with saccharose it continued in small amounts throughout the entire dehydration period (figs. 1 and 2). This uniformly marked loss of water

[3] Centrifuged for one-half hour at 3000 revolutions each minute.

was accompanied by corresponding decrease in body weight. In the series of experiments with saccharose the average water loss during the injection period and the subsequent three to five hours amounted to 9.7 per cent of the original body weight or 97 cc. for each kilogram. The

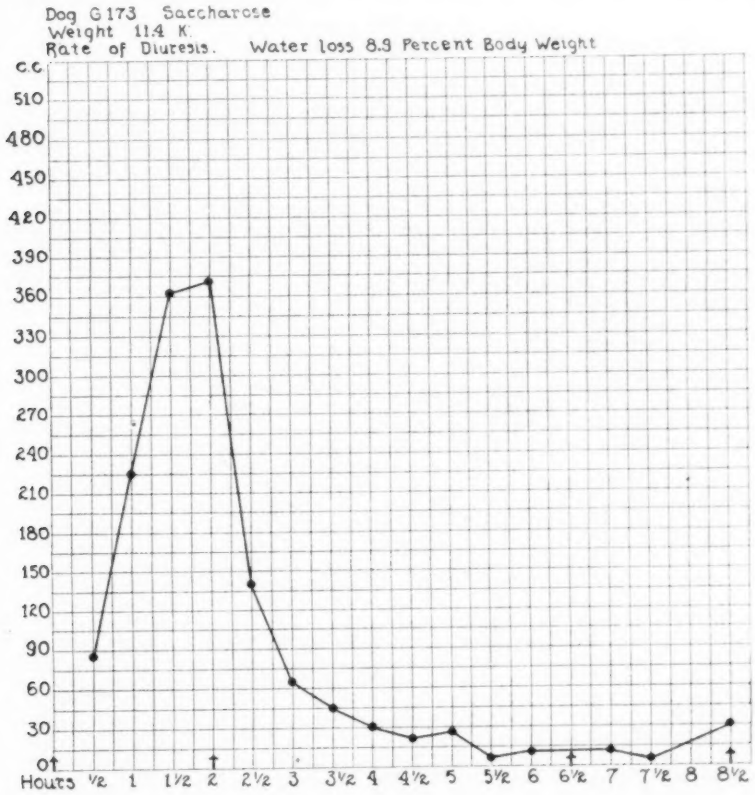

Fig. 1. Rate of diuresis with saccharose, 8 grams for each kilogram each hour Marked diuresis. Urine continuously excreted during time of experiment. Arrows indicate period of injection of saccharose, and of 0.05 per cent sodium chloride solution.

corresponding average actual loss of weight was 11.3 per cent. With glucose, the loss of water was slightly less, 7.7 per cent of the body weight, with an actual loss of weight or 10.5 per cent. The maximal rise in body temperature following such dehydration was 1.8° C. when saccharose was injected; with glucose it reached 3.4°. The latter rise was observed in

experiment G185, the animal having received only 25 cc. of water in the previous twenty-four hours. In none of the experiments was there a rise of temperature at the end of the injection, the rise occurring one-half hour to three hours later. In many of the experiments, the rise inbody temperature was less than 1° C. (table 3). With the administration of water

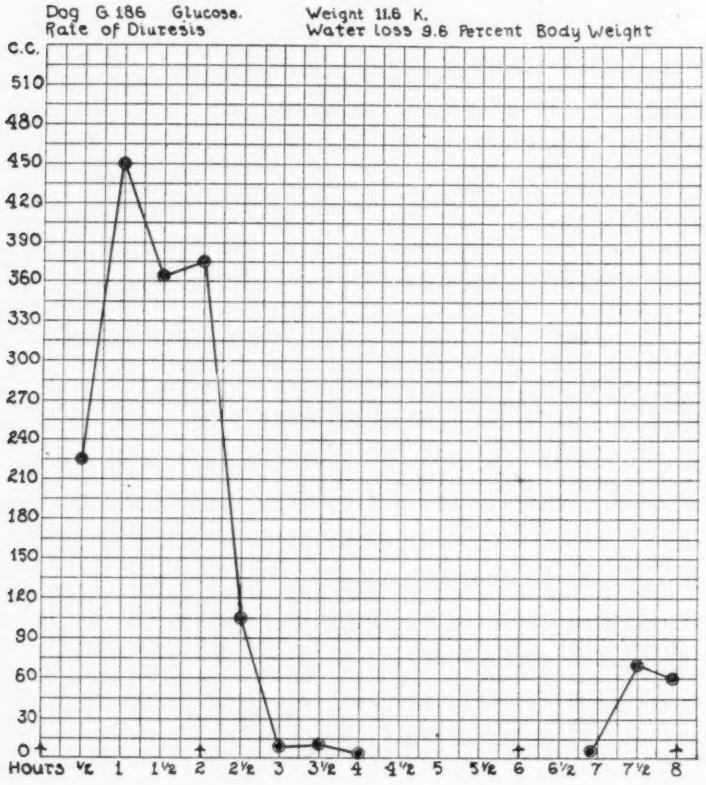

Fig. 2. Rate of diuresis with glucose, 9 grams for each kilogram each hour for two hours. Marked diuresis. Larger, initial output of urine with subsequent anuria. Reëstablishment of urine excretion after injection of 0.5 per cent sodium chlorid.

by mouth or of sodium chloride solution, intravenously, there was a slight fall in body temperature. The temperature curve in a typical saccharose and glucose experiment is shown in figures 3 and 4.

The next logical step was to ascertain whether an equivalent amount of water given to these dehydrated animals would be retained and result in a

corresponding gain in weight. Because of vomiting it was difficult to measure accurately the water given by mouth, but it was accomplished in nine experiments over a twenty-four-hour period (table 4). The weight of the water retained was found to correspond closely to the actual in-

TABLE 3

Loss of weight, dehydration period

DOG	WEIGHT, KGM.	LOSS OF BODY WEIGHT		LOSS OF URINE		TEMPERATURE VARIATIONS 0°C. RECTAL
		Kgm.	Per cent	Body weight, per cent	Cc. for each kgm.	
Saccharose						
F206 1	10.5	0.9	8.6	9.3	93	39.3 to 39.9
F206 2	10.4	1.1	10.7	10.7	107	38.8 to 38.9
F293 (1)	17.0	2.25	12.6	10.5	105	38.9 to 38.8
F550	9.1	1.3	14.0	10.5	105	39.8 to 39.2
E712	8.6	1.1	13.4	8.5	85	38.3 to 37.7
F616	9.8	0.9	9.2	9.4	94	39.4 to 40.2
F617	9.7	0.9	9.3	9.7	97	39.6 to 39.1
F685	11.0	1.4	12.7	9.6	96	38.9 to 38.4
F912	14.5	1.7	11.5	10.0	100	39.4 to 40.1
F914	10.7	1.2	11.2	9.6	96	38.8 to 39.6
G173	11.4	1.3	11.4	8.9	89	38.8 to 39.0
G176	8.9			10.4	104	39.0 to 40.8
Average			11.3	9.76	97.6	Maximal rise 1.8°
Glucose						
F918	13.2	1.4	10.6	7.0	70	38.8 to 39.3
G101	13.4	1.6	11.9	7.0	70	38.1 to 39.6
G170	18.1	1.7	9.5	6.5	65	38.8 to 41.8
G172	13.4	1.5	11.2	7.7	77	39.2 to 40.1
G181	11.6	1.3	11.2	9.6	96	38.8 to 39.8
G183	6.3	0.6	8.8	8.0	80	39.5 to 41.5
G185	10.8	1.1	10.5	8.2	82	39.3 to 42.7
Average			10.54	7.7	77	Maximal rise 3.4°

crease in body weight. In order to learn whether more rapid administration of water would afford a like result, four animals were given a continuous intravenous injection of a similar amount of a 0.5 per cent sodium chloride solution[4] during a period of two hours. The measured increase

[4] A solution of this strength was used because it contained a very small amount of sodium chlorid and yet did not produce hemolysis either grossly in the animal, or when fragility tests were carried out in vitro.

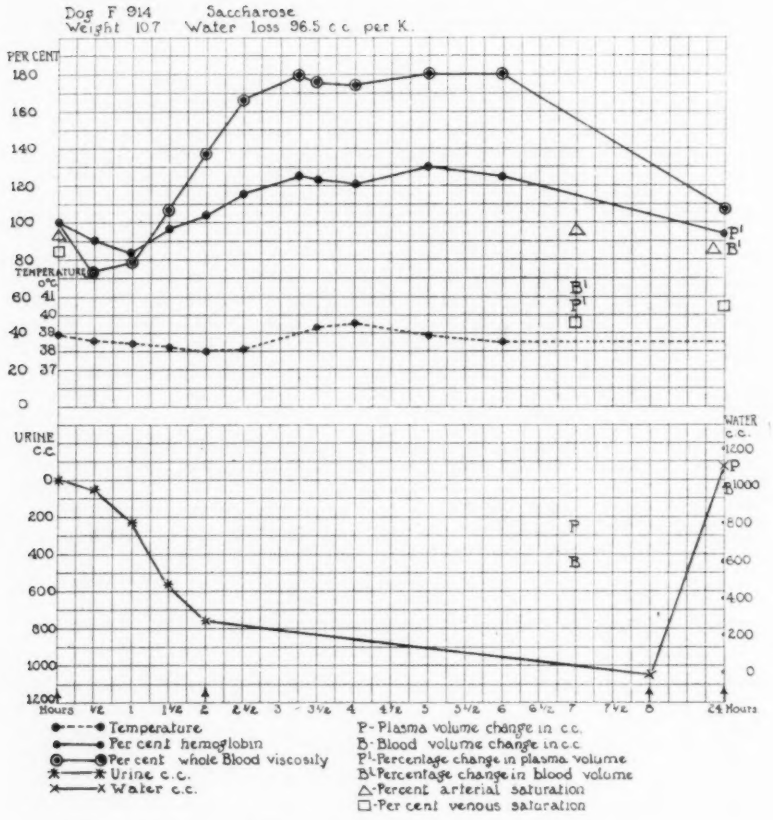

Fig. 3. Findings in the blood and urine following injection of 50 per cent saccharose solution, 8 grams for each kilogram each hour for two hours. Initial fall and then marked rise in blood viscosity and hemoglobin percentage; and fall in blood and plasma volume. With ingestion of water there is a marked fall in blood viscosity and hemoglobin percentage. (Arterial and venous oxygen saturation is also charted before, during and after dehydration.) During the entire experiment changes in body temperature were practically negligible. Note: *——* indicates c.c. of urine lost minus fluid intake.

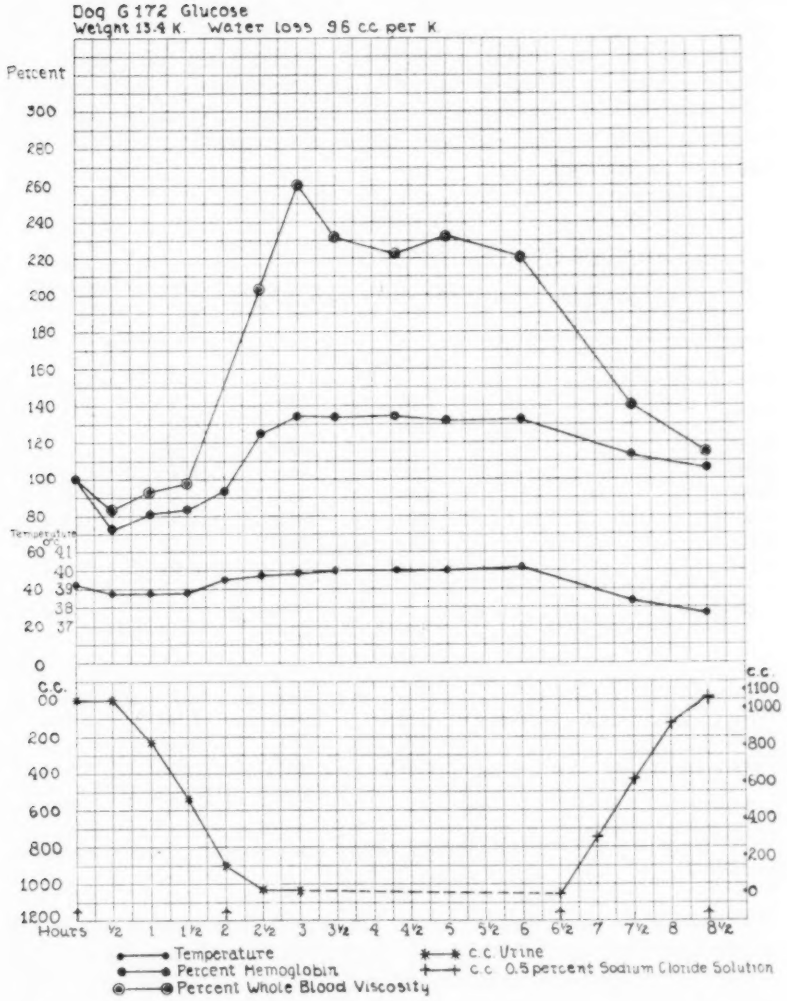

Fig. 4. Findings in the blood and urine following injection of 50 per cent glucose solution, 9 grams for each kilogram each hour for two hours. Initial fall and then marked rise in blood viscosity and hemoglobin percentage. With intravenous injection of 0.5 per cent sodium chloride solution there is a rapid fall in blood viscosity and hemoglobin percentage. During the entire experiment changes in body temperature were slight. Note: *——* indicates c.c. of urine lost minus fluid intake.

in weight again was accounted for by the amount of water retained. The average increase in percentage of body weight was 9.4 when saccharose was used and 7.9 with glucose. That water thus given to a dehydrated animal was almost immediately absorbed by the tissues was clearly shown in three experiments in which sodium chloride solution was administered intravenously. The injection invariably continued for from forty to fifty minutes before a measurable amount of urine began to be excreted.

TABLE 4

Increase in weight, restoration period

DOG	WATER INTAKE, CC.	WATER RETAINED, CC.	INCREASE IN BODY WEIGHT	
			Kgm.	Per cent
Saccharose				
F206 1	1800	1250	1.2	12.5
F206 2	1100	575	0.6	6.4
F293	1700	1470	1.05	7.0
F550	1000	860	0.8	10.2
F685	1000	997	0.8	8.3
F912	1700	1695	1.45	11.3
E712	1150	700	0.75	10.1
G173	1118*	1063	0.99	9.8
Average				9.4
Glucose				
F918	1100	955	0.7	6.0
G172	1110*	1060	1.05	8.8
G183	600*	570	0.43	7.5
G185	1006*	889	0.91	9.4
Average				7.9

*0.5 per cent sodium chlorid solution injected intravenously. Injection given in two hours.

Changes in the blood and circulation. During the period of injection and for the following three to five hours, the hemoglobin and viscosity in a unit volume of whole blood were determined at short regular intervals. This curve showed a close parallelism between the changes in hemoglobin concentration and viscosity (figs. 3 and 4). There was an early fall in both hemoglobin and viscosity, but later a marked and rapid rise which remained practically constant until the end of the dehydration period. In two experiments total blood and plasma volume determinations were also made during the injection for the purpose of determining whether the decreased

viscosity of 9 to 17 per cent and hemoglobin percentage of 10 to 20, were due to circulating volume changes. The determinations showed an increase of 9 to 23 per cent in total plasma, but only the slight rise of 3 to 6 per cent in whole blood. Thus, the decreased hemoglobin concentration, and the decreased viscosity, were shown to be accompanied by a corresponding quantitative increase in total plasma.

TABLE 5

Percentage changes in hemoglobin and whole blood viscosity. Estimations in control period 100 per cent

DOG	DEHYDRATION PERIOD		RESTORATION PERIOD	
	Hemoglobin, per cent	Whole blood viscosity, per cent	Hemoglobin, per cent	Whole blood viscosity, per cent
Saccharose				
F206 1	113	221	82	94
F206 2	128	211	104	119
F293	128	214	95	114
F550	121	192	92	102
E712	123	193	97	107
F616	120	170		109
F617	119	136	101	98
F685	119	134	100	85
F912		179		88
F914	125	180	94	109
G173*	138	244	92	91
G176*	140	210	96	89
Average	125	190	95	100
Glucose				
F918	119	170	93	100
G101	125	173	104	116
G170	126	208	102	120
G172*	132	220	108	114
G181*	131	180	93	97
G183*	126	232	91	88
G185*	130	208	100	96
Average	127	199	99	104

* 0.5 per cent sodium chlorid solution injected intravenously.
Injection given in two hours.

The blood and circulatory changes observed during the dehydration period were characteristic and remarkably constant. The venous blood was more difficult to obtain, darker in color, and appeared to clot more rapidly than normal. The hemoglobin rose 13 to 40 per cent. The viscosity of the

whole blood, plasma and serum revealed a definite constant rise (tables 5 and 6). The increase in viscosity of the whole blood averaged 95 per cent, in the plasma 23 per cent, and in the serum 27. The percentage decrease in plasma volume varied from 23 to 49, and the blood volume from 2 to 38. In four experiments, the chloride content of the plasma rose from 9 to 15 per cent (table 7). That there was a like concentration of the blood in the large veins and arteries was evidenced by simultaneous identical values for arterial and venous whole blood viscosity in two experiments. Determina-

TABLE 6

Viscosity changes

DOG	CONTROL PERIOD			DEHYDRATION PERIOD						RESTORATION PERIOD					
	Whole blood	Plasma	Serum	Whole blood	Per cent	Plasma	Per cent	Serum	Per cent	Whole blood	Per cent	Plasma	Per cent	Serum	Per cent
Saccharose															
F206 1	5.6	2.2	1.65	12.4	221	2.4	109	2.35	142	5.3	94	2.0	91	1.8	109
F206 2	5.45	1.75	1.65	11.5	211	2.2	126	2.05	124	6.5	119	2.1	120	1.9	115
F293	5.6	2.0	1.7	12.0	214	2.55	128	2.3	135	6.4	114	2.1	105	1.9	112
F550	5.3	2.2	1.75	10.2	192	3.1	141	2.4	137	5.4	102	2.5	118	1.8	103
F616	4.6	1.8	1.75	7.8	170	2.2	122	2.0	111	5.0	109	2.2	122	1.8	103
F617	4.7	1.75	1.65	6.4	136	2.0	114	1.9	115	4.6	98	1.95	111	1.75	106
F685	5.3			7.1	134					4.5	85				
F912	4.2			7.5*	179					3.7	88				
F914	4.6			8.3	180					5.0	109				
E712	6.0	1.8	1.6	11.6†	193	2.2	122	2.0	125	5.6	107	1.95	108	1.7	106
Glucose															
G185	5.3		1.5	11.0	208			1.9	127	5.1	96				
Average		1.9	1.7			2.4	123	2.1	127			2.1	111	1.8	108

* Arterial whole blood viscosity 7.4.

† Arterial whole blood viscosity 11.4.

tions of the oxygen content of arterial and venous blood were made in four experiments as a part of the study of the circulatory changes accompanying this degree of blood concentration. The results showed that the percentage saturation of oxygen in the arterial blood did not change significantly from the normal, while the venous oxygen saturation was definitely decreased (table 8). Noteworthy changes in the pulse rate were not observed. Direct systolic blood pressure determinations in three experiments did not reveal deviation from the normal. (Note tracing obtained in experiment F914, fig. 5.)

TABLE 7

Percentage changes in body weight, hemoglobin, plasma volume, blood volume and plasma chlorid concentration. Estimations in control period 100 per cent

DOG	BODY WEIGHT	HEMOGLOBIN	PLASMA VOLUME	BLOOD VOLUME	PLASMA CHLORIDS
Dehydration period					
F293	87.3	128	57	66.6	111
F550	85.7	121	73	88.0	
E712	86.6	123	51	61.0	
F616	90.8	120	65	75.0	
F617	90.7	119	76	82.0	109
F685	87.3	119	62	75.4	
F914	88.3	125	56	62.0	
F912			77	98.0	
G183	91.2	126			115
G185	89.5	130			114
Average	88.6	123	64	76.0	
Restoration period					
F293	93.5	95	100	100.0	
F550	94.5	92	92	92.0	100
E712	95.3	97	76	75.0	
F617	98.0	101	106	100.0	95
F685	94.5	100	86	84.0	
F914	96.3	94	95	87.0	
F912			111	98.0	
G183	98.0	91			
G185	98.0	100			
Average	96.0	96	95	91.0	

TABLE 8

Oxygen saturation of arterial and venous blood

DOG	CONTROL PERIOD		DEHYDRATION PERIOD		RESTORATION PERIOD	
	Arterial, per cent	Venous, per cent	Arterial, per cent	Venous, per cent	Arterial, per cent	Venous, per cent
F616	95	58	90.5	26.5		
F617			92.0	42.0		
E712	102	55	89.0	40.0		
F914	95	84	95.0	48.0	90	54

Following the retention of an adequate amount of water in the dehydrated animals, the blood and circulation were rapidly restored to a condition approximately normal. The actual hemoglobin and viscosity readings varied from 82 to 120 per cent of the original control findings, the average reading for hemoglobin being 97, whole blood viscosity 102, plasma viscosity 111, and serum viscosity 108 per cent. The restoration of plasma and blood volume was definite, although the deviations from the original normal values showed greater variations than the hemoglobin and viscosity. The actual plasma volumes varied from 76 to 111 per cent, but averaged 95 per cent, while the total blood volumes ranged from 75 to 100 per cent, averaging 91 per cent. In the single experiment

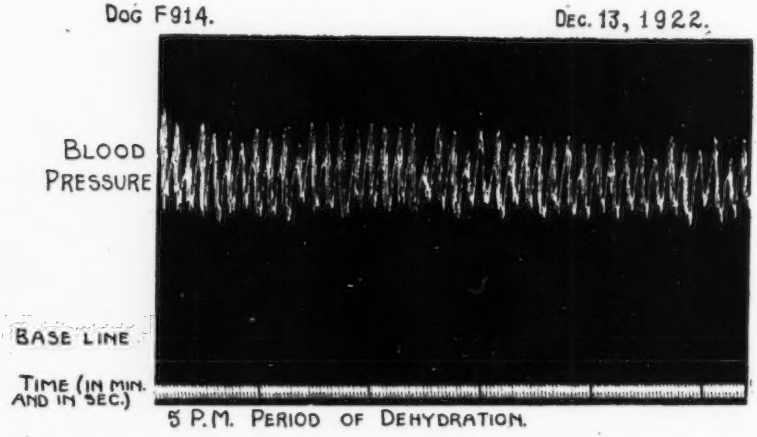

Fig. 5. Blood pressure 90 to 114 mm. Hg taken at 5 p.m., five hours after the injection of saccharose solution. Loss of weight 1.2 kg. (11.2 per cent). Water loss 96 cc. for each kilogram. Hemoglobin 125 per cent, whole blood viscosity 180 per cent, plasma volume 56 per cent, blood volume 62 per cent of determinations made in normal control period.

(F914), in which the venous oxygen saturation was estimated in this restoration period, there was a rise of 6 per cent from that found during dehydration. Similarly an increased plasma chloride content fell in two experiments to the normal control level (table 7).

Discussion. The symptoms exhibited by the dehydrated animals are in many respects the same as those of patients who have lost a large amount of fluid rapidly. The thirst is undoubtedly due to the concentration of the body fluids. Vomiting is characteristic of all conditions in which the circulating blood volume is reduced rapidly.

Prostration and collapse which are always strikingly shown in cases of Asiatic cholera, severe bacillary dysentery and acute anhydremia, would

undoubtedly have occurred in these experiments, had water not been administered early in the period of dehydration. Additional experiments in which the dehydration period is further prolonged should throw light on the important therapeutic problem of when and in what manner fluid should be administered to dehydrated patients.

The symptoms of restlessness, muscular twitchings and hyperpnea were transitory and infrequent. They occurred just after the completion of the injections and were probably caused by the concentration of the saccharose or glucose in the nervous tissues as well as by loss of available water.

The experiments afforded an accurate demonstration of very rapid dehydration; 8 to 10 per cent of the animal's entire store of water was excreted in a few hours. As a result, definite changes in the circulation and in metabolism occurred; but if the dehydration period was not too prolonged the animal recovered quickly on the administration of a sufficient amount of water. Tobler (12) produced severe rapid dehydration in puppies by causing diarrhea with large doses of magnesium sulphate, but the dehydration extended for from three and one-half to seven days.

The results of the experiments prove that a dog with normal water reserve can lose rapidly an amount of water equivalent to 7 to 10 per cent of the body weight without a rise in body temperature. When the normal reserve has been depleted as in experiment G185, a rise of 3.4 degrees did occur. This maximal rise in body temperature is in contrast to the marked rises observed in similar experiments by Balcar, Sansum and Woodyatt (1). In their experiments, the intake of glucose was much greater in a given time, and the ratio of water intake to water output was 1 to 2 or less, with a maximal rise of temperature to 23.6° F., while in the present series, the water ratio was 1 to 3 with no appreciable rise of temperature. The development of hyperthermia in this type of experiment would thus appear to depend on the actual amount of substance injected, and the ratio of water intake to water ouput.

The blood and circulatory changes with rapid experimental dehydration are largely dependent on diminished circulating blood volume. The decreased blood volume is itself due to a diminished volume of plasma, which in turn is the result of water loss. In experimental and clinical conditions in which the blood volume is decreased, the volume flow is, as a rule, diminished. No direct determinations of blood flow were made in these experiments, but the finding of normal arterial oxygen saturation, together with a lowered oxygen saturation in the venous blood, would indicate that the volume flow was decreased. The blood changes thus outlined are also characteristic of surgical or wound shock. But in shock there may be no rise in the protein concentration of the plasma. This difference in the protein concentration of the plasma may be one of the reasons why, in acute dehydration, the systolic blood pressure is normal, and in shock, usually low.

Marriott (7) has reviewed the blood changes, including concentration, that have been observed by pediatricians in the acute anhydremia of infants. Rogers (10) and others report a highly concentrated blood in Asiatic cholera. In these conditions, the dehydration results chiefly from excessive water loss by way of the stools. Dehydration also occurs clinically from loss of fluid by way of the stomach, either by continued vomiting, as in pernicious vomiting of pregnancy, or by marked excretion with retentionas in cases of duodenal obstruction.[2] In the present experiments marked loss of water has followed extreme diuresis with blood changes similar to those in the above clinical conditions, but no infectious or known toxic causative factors were employed in the production of the experimental dehydration.

The rapid restoration of the circulation on the administration of water was striking in these experiments thus showing that lack of water is accountable for the circulatory disturbances. It further emphasizes the importance of early therapeutic administration of water in all conditions in which dehydration is present.

SUMMARY

The intravenous administration of saccharose or glucose in the amount and manner employed in these experiments offers a simple method for studying the effects of rapid dehydration in the living animal.

From 7 to 10 per cent of the animal's entire store of water was excreted in a few hours, without fatal results. With this degree of dehydration there was no significant change in body temperature.

A study of the blood and circulation after the production of experimental dehydration revealed increased viscosity and hemoglobin concentration, distinctly diminished volume of plasma, but normal arterial blood pressure.

There was also indirect evidence of diminished blood volume flow.

The blood and circulation were rapidly restored to normal if the aminal was given an adequate amount of water within a few hours of the onset of dehydration.

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VITAMIN-A AND EXERCISE IN RELATION TO FOLLICULAR ATRESIA IN THE OPOSSUM

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Received for publication December 14, 1923

About nine years ago the writer began the study of the embryology of the opossum and during the last three years has been especially interested in the oestrous cycle and various phases in the physiology of reproduction of this marsupial.

During the first few years little attention was paid to the diet. This consisted of skim milk, bread, tallow and lean meat. But during the season 1916-1917, when the colony rose to some 250 animals,[1] the problem of cheap feeding was solved by securing slaughter-house offal, such as "lights," heart, liver and tripe.

It is of interest to note that before feeding entrails a number of animals each year developed an exceedingly repulsive infection of the eyeball, which must doubtless be referred to xerophthalmia. Almost no eye trouble has since been encountered except sporadic cases of slight opacity of the eyeball, usually of one eye only.[2]

There has persisted, however, a type of sterility characterized by atresia of the ovarian follicles, which has been noted for several years in animals kept some months in the cages. The trouble seemed to grow progressively worse the longer the animals were confined in close quarters, although many animals recovered spontaneously. In the sterile animals the typical oestrous changes usually either disappeared altogether or the cycles became greatly prolonged and were then abnormal in their manifestations.

During the collecting season of 1922[3] Prof. H. M. Evans suggested to me a possible relation between the diet of the animals and their sterility, for in collaboration with Dr. Katherine Scott Bishop (2), (8) he had discovered a type of sterility in the rat referable to a deficiency in vitamin-A too light to affect the weight and general "health" of the animal but yet sufficient to cause an upset in the oestrous cycle. Biology was thus given a most delicate "indicator" for vitamin-A.

[1] Expenses at this time borne by the Wistar Initute of Anatomy and Biology, Philadelphia, Dr. M. J. Greenman, Director.

[2] See Hartman (3a), figure 3, plate 1, for a photograph of such an animal.

[3] Expenses borne by the Bache Fund, American Academy of Science, Dr. R. G. Harrison, Chairman.

Other workers have also noted the relation of the dietary deficiency or inanition to sterility. Reynolds and Macomber (5), (6) were able to record sterility as an effect of a diet deficient in vitamin-A, in proteins, in calcium salts or in all of these food essentials. These authors mention follicular atresia and figure an early stage in the process; but apparently they did not see the profound and wholesale destruction of ovarian follicles occurring in the opossum (see accompanying plate of figures). Leo Loeb (4) also noted atresia and ovarian hypofunction as a result of underfeeding. Weak and palpably "unhealthy" animals are likely to have defective ovaries, hence fail to exhibit oestrous changes of the reproductive organs, as I have observed in hundreds of cases. Stieve (9), Schiller (7), Ceni (1), and many others have called attention to the sensitiveness of the germ cells to noxious stimuli. Death of the female germ cells means, of course, atresia of the follicles that contain them. The experiments of Evans and Bishop (2) are especially instructive in this regard.

It was therefore decided to determine, if possible, whether the type of follicular atresia encountered in my captive opossums was due also to a dietary defect, more especially to a lack of fat-soluble vitamin.

Some 65 female opossums were used in the test. These animals had been followed through oestrous cycles from the middle of January on. The animals were killed at oestrus and at autopsy the ovaries were examined and, if ovulation had taken place, the eggs were recovered from the fallopian tubes or uteri. Oestrus was diagnosed by Stockard and Papanicolaou's (10) vaginal smear method. Often the vaginal content would be somewhat abnormal in correlation with abnormal ovaries within. Animals were considered "normal" if the ovarian follicles were clear or if ovulation had taken place. Atresia of the follicles is manifested in the earliest stage by a cloudiness of the liquor folliculi. Microscopic sections of material fixed in Meves' fluid show strikingly the fatty degeneration of the membrana granulosa as may be seen in the photographs on accompanying plate.

The basic diet fed all animals consisted of stale bread soaked in separated milk, lean meat and tallow or "cracklings." About once a week entrails were fed instead of meat, except to nos. 847, 863 and 783 of the table. No limit was placed on the bread and skim milk of which there was always an over-abundance available. The animals receiving nothing but the basic diet are listed in column 11 of the accompanying table of results. Five animals (column 9) received 4.5 grams of Fleischmann's yeast per kilo of body weight and 22.5 grams of best canned tomatoes. Five other animals (column 10) received only the yeast in addition to the basic diet.

Columns 9, 10 and 11 of the table therefore record control animals.

Since in this experiment attention is directed especially to vitamin-A, 25 animals were given daily 60 cc. of whole milk per kilo of body weight. Ten animals received yeast and tomatoes also. But after March 27 4 cc. of

TABLE 1

Giving results of feeding experiments. The numbers (column 1) refer to the forty individuals killed on the date mentioned (column 2). In column 3 (Atresia I) cases are recorded in which the ovarian follicles had remained of medium size; cases are entered in column 4 if the follicles had attained a larger size (Atresia II). For other details see text

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
NUMBER	DATE	CONDITION			VITAMINS ADDED TO BASIC DIET						REMARKS
		Atresia		Normal	$A^{+++}BC$	A^{+++}	A^{+}	BC	B	C	
		I	II								
689	Feb. 18	*								*	
877a	Feb. 22	*					*				15 days
821	Mch. 7			*						*	Ovulation
839	Mch. 9		*				*				36 days
820	Mch. 10		*							*	
713	Mch. 11	*						*			
741	Mch. 11	*						*			
747	Mch. 11		*				*				32 days
757	Mch. 11	*							*		
769	Mch. 11		*						*		
830	Mch. 11			*						*	Follicles clear
847	Mch. 13		*							*	
768	Mch. 14	*					*				3 days only
783	Mch. 14		*							*	
688	Mch. 14		*						*		
781	Mch. 14		*				*				35 days (figs. 3 and 4)
861	Mch. 24		*				*				45 days
755	Mch. 25			*						*	Ovulation
849	Mch. 26		*					*			
819	Apr. 1		*							*	
750	Apr. 5			*				*			Ovulation
753a	Apr. 5			*			*				Ovulation (25 days)
884a	Apr. 7			*				*			Ovulation
838	Apr. 8			*						*	Pregnant
863	Apr. 8			*						*	Ovulation
879	Apr. 8		*							*	
803	Apr. 11			*					*		
728	Apr. 22		*		*		*				A^{+}, 17 days; $A^{+++}BC$, 23 days
843	Apr. 22		*				*				74 days
822	Apr. 25	*			*		*				A^{+}, 10 days; $A^{+++}BC$, 14 days
705	Apr. 27		*		*		*				A^{+}, 17 days; $A^{+++}BC$, 30 days
799	Apr. 27		*						*		
800	Apr. 27		*			*	*				A^{+}, 17 days; A^{+++}, 16 days
891	Apr. 27		*		*						16 days
696[1]	May 1			*	*		*				A^{+}, 46 days; $A^{+++}BC$, 5 days
740[1]	May 2		*		*		*				A^{+}, 17 days; $A^{+++}BC$, 35 days (figs. 1 and 2)
751[1]	May 3			*		*	*				A^{+}, 31 days; A^{+++} 22 days Ovulation
752[1]	May 3			*	*		*				A^{+}, 17 days; $A^{+++}BC$, 36 days Ovulation
738[1]	May 10			*	*		*				A^{+}, 17 days; $A^{+++}BC$, 43 days Ovulation
706[1]	May 25				*		*				A^{+}, 17 days; $A^{+++}BC$, 40 days Resting stage

[1] No more Cod Liver Oil fed after April 26; animals turned loose in room.

cod liver oil per kilo were added to the whole milk to insure a sufficiency of vitamin-A. These animals were thus transferred from column 8 ("A+" or whole milk) of the table to columns 6 and 7 ("A+++" or whole milk plus cod liver oil).

It is certain, then, that a number of animals received an adequate supply of vitamin-A for a considerable period of time.

Omitting for the present the consideration of the last six animals listed, it is evident from an inspection of the table of results that the ability to ovulate is independent of the nutritional régimes employed in this experiment, for the distribution of cases showing normal sexual behavior is a random one. Ovulation is as likely to occur in animals receiving only the basic diet as in those receiving in addition to this, yeast, tomatoes, butter-fat and cod liver oil.

Whatever may be said of the diets used, it is certain that vitamin-A has nothing to do with the type of sterility by atresia of the ovarian follicles here described—butter-fat and cod liver oil fail to prevent the atresia of the follicles. It should be added that the animals used were for the most part as fine a lot of opossums in vigor, weight, sleekness of fur, etc., as I have ever had.

On April 26 a test for the effect of exercise was begun. Up to that date the animals were confined in cages $2 \times 2 \times 2$ feet, four to five animals to a cage. The remaining animals were now given the run of a room 12×14 feet in size. During the following night most of the animals escaped but there remained the last six mentioned in the table. All of these had been receiving cod liver oil in addition to whole milk for some time; but on April 26 the cod liver oil was discontinued. Four animals promptly ovulated. The figures are meager, but such as they are they point to the beneficial effects of exercise on fertility, which is in line with the observations of others.

It seems highly probable, from a consideration of the data presented above, that the sterility of the opossum through atresia of growing ovarian follicles is not due to faulty diet and it is certainly not due to lack of vitamin-A. The condition is, therefore, not comparable to that induced by Evans and Bishop in the rat by reducing the quantity of vitamin-A nor to the condition found by Reynolds and Macomber (5), (6). The trouble is doubtless due to cage life in which the lack of exercise plays an important part. It is rather comparable to the degeneration of ripe oöcytes of laying hens experimented on by Stieve (9), who removed the hens from the freedom of large runways to the close confinement of small cages, food remaining the same.

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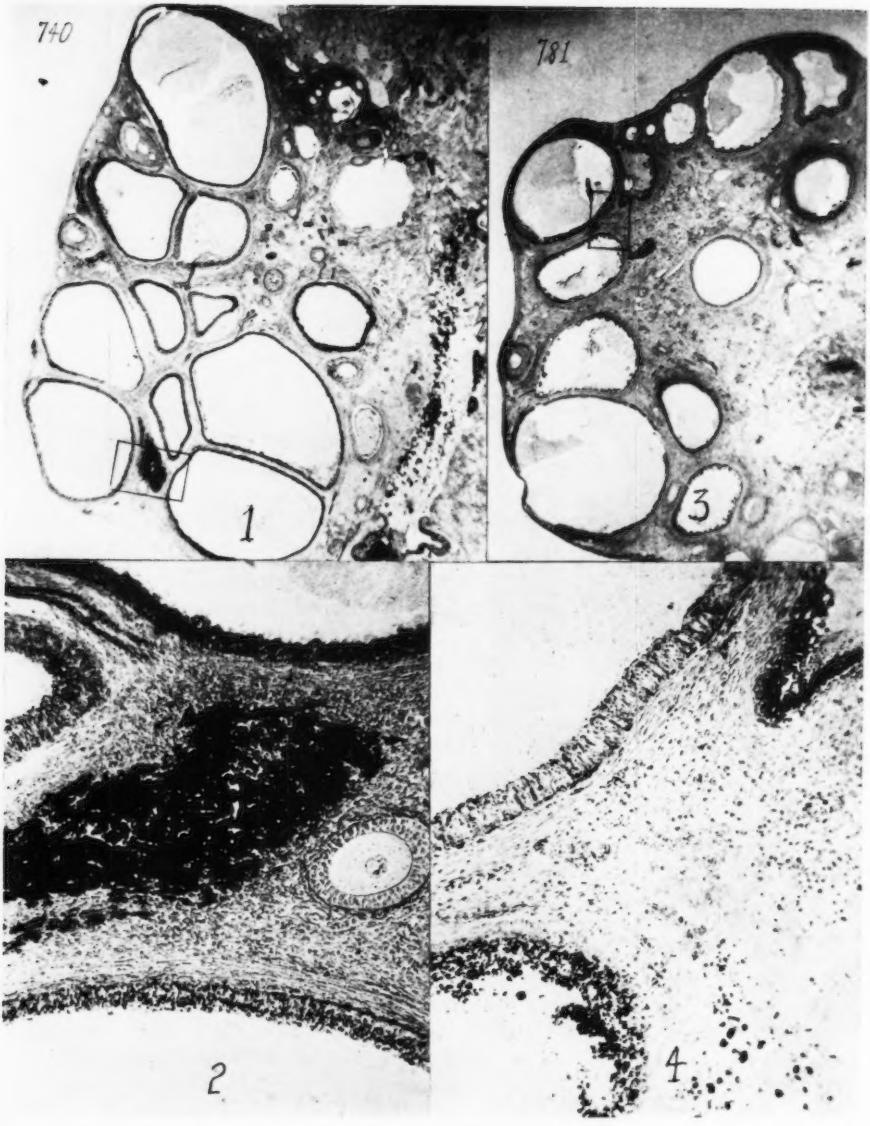

Fig. 1. Portion of ovary of no. 740. The Graafian folliclesare lined with granulosa cells undergoing atresia. Fat granules stained black with modified Flemming fluid. Saffranin stain. ×15.

Fig. 2. Detail from figure 1. In the center is a follicle in an advanced stage of atresia. ×100.

Fig. 3. Portion of ovary of no. 781. Flemming fixation.saffranin stain. ×15.

Fig. 4. Detail from figure 3. ×100.

THE EFFECT OF COLORED BACKGROUNDS ON AREA OF COLOR FIELDS

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Received for publication December 22, 1923

This investigation was undertaken to ascertain if the color quality of the background modified to any extent the area of the color field in the retina. Many experiments have been performed in the attempt to map the zones of color sensitiveness in the retina; these have been reviewed by Baird (1), who summarizes his findings somewhat as follows: "The zone of stable red is coextensive with that of stable green; the zone of stable yellow is coextensive with that of stable blue; the yellow-blue zone is much more widely extended in all directions than the red-green zone; the nasal side of the retina has the widest extension of color sensitivity, and there is wide individual variation in zonal extension."

These experiments of Baird were apparently all performed with an unvarying background. In 1913, Rand (2) performed a series of experiments to determine what factors of variation in the stimuli influence the sensitivity of the retina to color. She sought to ascertain what variants were introduced by: *a*, brightness of stimuli; *b*, brightness of field surrounding stimuli; *c*, brightness of preëxposure; *d*, general illumination of the retina. Concerning the first point she finds that relative brightness of stimuli has no effect on zonal extension provided the surrounding field is duly equated in brightness to the stimulus being tested. The second point most nearly touches our present problem. Rand used background screens of white, black and graded grays; she found: "For yellow the limen of sensitivity was lower with the white screen, but its limit was wider with the black screen. For green, the limen was lower and the limit was wider with the white than with the black screen. For red, the limen was lower and the limit was wider with the black than with the white screen. For blue, the limen was lower with the black screen but wider with the white screen" (p. 125). That is, a dark background somewhat widens the zone for perception of yellow and red, but a white background widens the zone for perception of green and blue. (*Cf. infra* our findings.) Concerning the third point, Rand found that whereas the effect of preëxposure was considerably modified by the rela-

tive brightness of either screen or stimulus, and might be either that of contrast or after image, the general alteration, whenever induced, was toward that of narrowing of field. Concerning the fourth point, general illumination, she found that without standardization and uniformity of illumination the findings of like experiments on successive days were unreliable and not comparable.

Profiting by the experiments of Rand, and using them as a point of departure, we sought to ascertain the relative extension of the retinal color zones under a set of conditions where the only variable factor for a given subject was the color of the background.

The work was carried on in a laboratory room from which all daylight was excluded by means of opaque screens. For constant illumination we used two 100-watt "daylight" Mazda bulbs suspended one meter above the center of the campimeter and one and a half meters distant. The campimeter used consisted of a rigid frame, with glass front, enclosing a heavy white cardboard on which was drawn a sector representing the upper left three-eighths of a circle; this sector was constructed with a total radius of 27 centimeters, with concentric arcs every 3 centimeters, and subdividing radii every 22.5 degrees. Twenty centimeters in front of the campimeter was a chin support so arranged with lateral motion that the optical axis of the subject's left eye could be brought to coincidence with the center of the sector. The upper border of the campimeter was so arranged to admit, between the glass and the described sector, thin transparent colored gelatine sheets; four such colored gelatine sheets were used, an ultramarine blue, a lemon yellow, a vermillion red and an emerald green; these sheets were so finely transparent that the underlying sector could be seen almost as clearly as when uncovered.

Stimuli sets were provided by mounting lengthwise on slender white sticks strips of colored paper 4 mm. wide by 12 mm. long. Four such stimuli were made (blue, red, green and yellow), carefully selected from the Bradley color papers to secure samples as free from admixtures as possible; they were each tested on twenty different individuals for spectral value, and were pronounced excellent and apparently free from admixture.

Method of procedure. The subject, with his right eye hoodwinked throughout the experiment, was comfortably seated on a stool, with his chin so placed on the chin-rest as to bring the optical axis of his left eye in coincidence with the center of the sector; he was strictly cautioned to maintain this coincidence undeviatingly; any disregard of this caution was immediately checked by the observer who, from his station just behind the subject, could note the reflection of the subject's eye in relation to the center of the sector. To avoid fatigue, and after image, the left eye of the subject was kept covered with black cardboard except at

the moment of decision. With these sundry precautions the fields of color vision, corresponding to the sector described, were mapped for each of the stimuli, first with a white background, then in succession with the blue background, the red and the green. Six subjects were studied, all volunteers from the class in Physiology at the University of North Dakota. From the mass of data thus obtained, the average mean was determined for each radius with each separate stimulus on each separate background, and the results charted; these results are shown graphically on plates 1 to 8 inclusive.

Figure 1 shows the four color fields when the background is white. It agrees with the usual finding except the green field seems unusually extended. It does not agree with Baird's conclusion that the yellow field is coextensive with the blue. Figures 2, 3 and 4 show the outer limits of the several color fields when the campimeter backgrounds are blue, red and green, respectively. But the several findings are brought out more sharply in the succeeding figures. Figure 5 shows how the outer limits of the blue color field vary according to the color of the background. It will be seen that its limits are more extended for both red and green than for white, but when its own color is used for a background, the color field for blue is contracted. Figure 6 indicates that the color field for red is greater when the background is either blue or green than when it is white. Figure 7 indicates that the color field for green is considerably contracted when blue is used for a background; but has about the same square area, though not the same outline, whether red or green or white is used for a background. Figure 8 indicates that the color field for yellow is most extended when red is used for a background; with not much departure in square area, though with considerable variation in contour, when blue, white or green backgrounds are used.

CONCLUSIONS

1. The extent and contour of the retinal field for the four colors, blue, red, green and yellow, vary with the background on which the stimulus color is observed.

2. The color field, when the stimulus color is observed on a like background, is much contracted with red, considerably contracted with blue, somewhat contracted with green.

3. The color field for either blue or yellow is greatest when red is the background; for red, when blue is the background; for green, when either white or green is the background.

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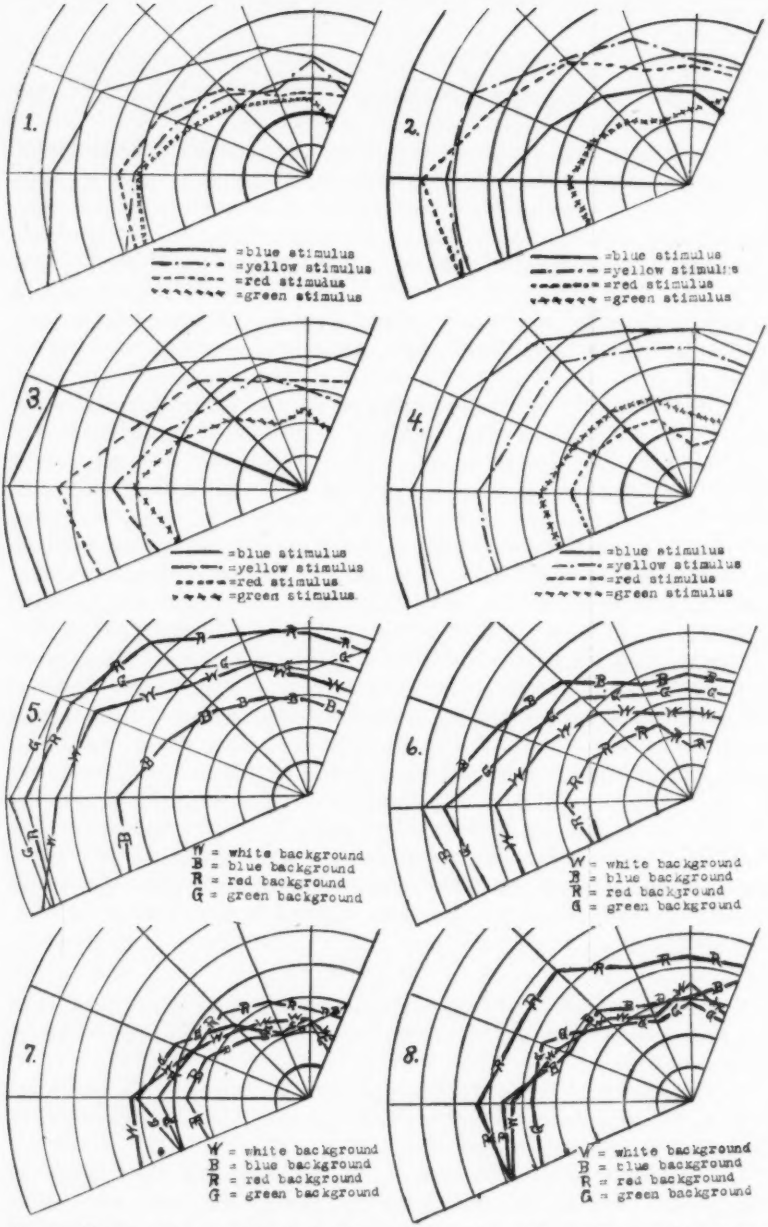

Fig. 1. Showing the outer limits in this retinal sector, of four different color fields when the campimeter back ground is WHITE.

Fig. 2. The same when the background is BLUE.

Fig. 3. The same when the background is GREEN.

Fig. 4. The same when the background is RED.

Fig. 5. Showing how the outer limits of the BLUE color field is altered by variation in the background color of the campimeter.

Fig. 6. The same for the RED color field.

Fig. 7. The same for the GREEN color field.

Fig. 8. The same for the YELLOW color field.

COMPETENCY OF THE URETER-VESICAL VALVES

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Received for publication January 14, 1924

The series of experiments here detailed were undertaken as a part of the investigation of the relationship of kidney involvement in prostatic hypertrophy. They consisted in subjecting the bladders of dogs to varying amounts of water pressure in an attempt to determine whether or not there was regurgitation into the ureter. Lucas (1) reported ten experiments where he used air pressure within the bladder, gradually increasing the pressure until the bladder ruptured. In no case did he get a reflux into the ureter.

In our experiments we used water pressure introduced into the bladder through an incision near the apex of the bladder, tightly closed about a glass tube, and varied this pressure to meet conditions.

Method. Morphine-chloretone anesthesia was used. When the dogs were sufficiently quiet, that is, in about two hours, the abdomen was opened and a glass tube inserted into the apex of the bladder and tightly tied there. This tube was connected with a bottle which, in turn, was mounted on a pulley in a graduated frame, allowing accurate placement of the bottle at heights varying from zero to 130 cm. The right ureter was severed at about its middle and at the distal end a cannula was introduced. The left kidney was placed in an oncometer and connected with a membrane manometer registering on a moving drum. The fluid used was normal salt so as to avoid any irritation effects. The pressure bottle was then placed at varying heights for different intervals of time, as shown in figure 1. At no time was there any escape of fluid into the ureteral cannula. The oncometer records were practically negative, indicating that in the normal bladder pressure variation does not produce any marked effect.

The question might be raised as to the transference of these results, which were obtained on dogs, to those on man, but Kelly and Burnham (2) state: "the lower end of the ureter of dogs strongly suggests the arrangement found in men;" and Macht (3): "the histological structure of the urethra and bladder *in dogs is practically the same* as that in man."

CONCLUSIONS

From our experiments, and those of Lucas, it would seem that in the normal bladder the uretero-vesical valves are competent to resist any intra-vesical pressure that is liable to occur.

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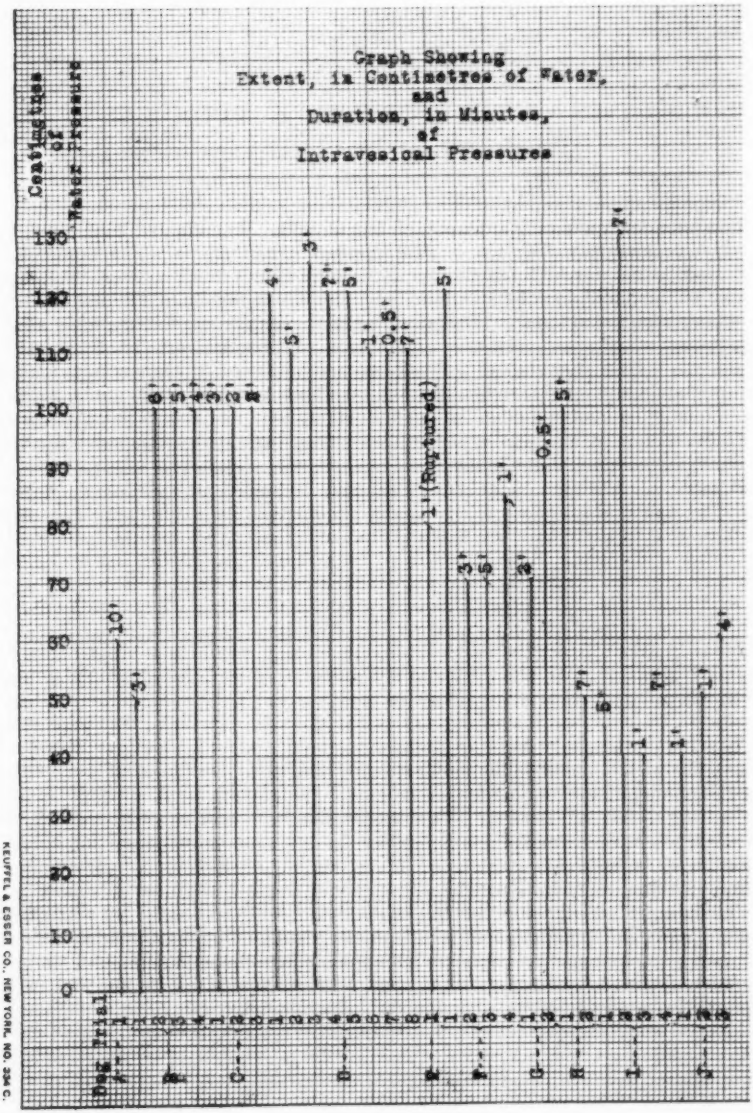
Graph Showing
Extent, in Centimetres of Water,
and
Duration, in Minutes,
of
Intravesical Pressures
Centimetres of Water Pressure
130
120
110
100
90
80
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0
10'
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2'
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24'
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2'
2'
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KEUFFEL & ESSER CO., NEW YORK, NO. 334 C.

Fig. 1

THIRTY-SIXTH ANNUAL MEETING

St. Louis, Missouri, December 27, 28, 29, 1923

Relation of oxygen to the growth of tissue cells. MONTROSE T. BURROWS.

It has been found that normal digestion, syntheses as well as migration of body cells, depends on the accumulation about them of certain blood-soluble products formed by them only in the presence of oxygen. Autolytic products are toxic. The stimulating products are formed only in the presence of oxygen and their formation is proportional to the oxygen present. This stimulating substance or substances in high concentration causes a digestion of the cells together with proteins and fats in the culture medium. In lower concentration fats disappear in the medium, the cells grow and divide. In still lower concentration migration alone and function (rhythmical muscular contraction) is induced. The cells digested in the presence of a high concentration of this stimulating substance or substances are food for other cells. These products in high concentration cause a digestion of the cells histologically similar to autolysis resulting from the absence of oxygen. This stimulus or stimuli may be readily washed away in the culture. The actively growing embryonic tissues, the tissues of cancer, wounds and the nail, have a poor circulation. The circulation in these tissues in either sinusoidal or the capillaries are few in number per unit cell area. A study of the substances and conditions which lead to cancer, such as coal tar, other lipoid solvents, certain congenital tumors, chronic inflammation, lead either directly or indirectly to build conditions suitable for such an accumulation of the growth stimulus about the cells. They build a densely cellular tissue relatively poor in blood vessels or a tissue in which the capillaries are dilated and the circulation within them is sluggish. Studies of tissue cultures have shown that stagnation and crowding leads to growth. Scattered cells and cells well washed are preserved and become inactive.

Ether in blood. T. K. KRUSE.

Dogs were rapidly anesthetized with ether and connected by a tracheal cannula to an automatic ether chamber provided with Guthrie valves. The carotid artery was exposed for obtaining blood samples. At various intervals air containing a mixture of inspired and expired air was withdrawn from the tracheal cannula and its ether content determined. At nearly the same time an arterial blood and an alveolar air sample were taken and their ether content determined. The airs were analyzed by differential absorption in concentrated sulphuric acid and the blood sample by the method described below.

One to 2 cc. of blood were put into a 30 cc. test tube containing a 1 per cent tannic acid solution. The test tube was provided with an aspirator attachment and connected with a receiver. The ether in the blood mixture was boiled out and was collected in the receiver. The resulting

ether-air mixture was transferred to an analyzer and its ether content determined.

The same procedure is suitable for the determination of ethylene in blood, even in the presence of ether. The absorber used for ethylene is a 100 per cent mercuric nitrate solution in nitric acid. In case both ether and ethylene are present, the gas is first passed into the sulphuric acid absorber to remove the ether and then into the mercuric nitrate absorber to remove the ethylene.

It was found that the theoretical equilibrium of ether contained in inspired air and blood does not readily take place, particularly early in anesthesia when the animal is in good condition. In the last hours of a prolonged anesthesia when the animal is in a reduced state the equilibrium occurs more readily. For instance, during an experiment a dog inspired 15 to 4.5 per cent of ether (average 6 to 7 per cent) for an hour. The blood contained 24 volumes per cent of ether and the alveolar air 2.8 per cent. After 3 hours of anesthesia at a time when the animal was lightly anesthetized, administration of 9 to 5 per cent of ether (average 6 to 7 per cent) for an hour, the blood contained 36 volumes per cent of ether and the alveolar air 4.6 per cent, an increase of over 50 per cent in both, although the administration of ether was similar. This demonstrates that in the later stages of anesthesia more ether is found in the blood by a similar treatment than in earlier stages. These results are not in harmony quantitatively with the laws of simple diffusion and require consideration of physiological factors, such as circulation and the functional state of the tissues.

Changes in the concentration of the blood following the administration of excessive quantities of water. C. H. Greene and L. G. Rowntree.

The administration of large amounts of water by mouth to dogs or other laboratory animals produces severe intoxication that may lead to convulsions and death. These effects of excess of water alone have been studied and the details are as follows:

Water was given to dogs by stomach tube, in quantities of 5 per cent of the body weight, at half-hourly intervals. In an experiment of eight hours' duration an amount of water equal to 80 per cent of the initial weight of the animal was administered. In the presence of such an excess an appreciable amount of water was retained in the body. We present the distribution and effect of the retained water with especial reference to the changes produced in the blood, which as a whole shows definite evidence of dilution. Changes in the hemoglobin in man were not found by Haldane and Priestley even after drinking 5 liters of water during a period of two hours, though they did find a slight decrease in the conductivity of the blood serum. In dogs, forced administration of water leads to a decrease in the hemoglobin. This is most rapid during the first two hours, but continues throughout the experiment. The degree of change varies, but usually is about 15 per cent.

The circulating plasma volume, determined by the dye method of Keith, Rowntree and Geraghty, is increased during the period of diminished hemoglobin concentration. The physico-chemical constitution of the blood serum was studied in detail. The concentration of protein in the serum was determined by the refractive index, the relative viscosity

and the surface tension. The changes in the serum proteins are similar in character and magnitude to those in the hemoglobin.

The salt content of the serum as determined by the electrical conductivity and the depression of the freezing point is reduced. The concentration of the chloride and sodium ions in the serum decreases to a much greater extent than that expected from the degree of dilution. In individual experiments, the plasma chlorides dropped to as low as 411 mgm. of sodium chloride in 100 cc. The average decrease in the chlorides varied between 20 and 30 per cent, a change definitely greater than that in the hemoglobin. The sodium concentration in the serum varies with the chlorides.

The changes in the hemoglobin and the serum proteins all show dilution of the blood and are indicative of a true hydremia. Comparison of the refractive index and of the serum viscosity indicates there is no change in the relative proportion of the various serum proteins. On the other hand the changes in the chlorides are greater than may be accounted for by dilution alone. The possibility of a "washing out" of the chlorides is suggested by the diuresis observed in some of the experiments. Diuresis is not invariable and the decrease in the blood chlorides is quite as marked in the animals with oliguria or anuria as in those with the most marked diuresis. The loss of chlorides through the urine is slight.

Frank edema does not appear under the conditions of the experiment. There is evidence of changes in the tissue as great as those in the blood, a water effect now being studied in detail.

Metabolic studies in experimental dehydration. NORMAN M. KEITH.

Dehydration was produced by the continuous intravenous injection of hypertonic saccharose or glucose solutions. A 50 per cent solution was administered to healthy dogs, the actual amount given was 8 to 9 grams for each kilogram of body weight, each hour for two hours. The resulting loss of water amounted to from 7 to 10 per cent of the body weight. Water or 0.5 per cent sodium chloride solution equal to that lost was then given to the dehydrated animal. The urinary output and blood concentration of glucose ,urea and chlorids were determined during the control, dehydration, and restoration periods.

The amount of glucose excreted in the urine was 29 to 37 per cent of that injected, and the concentration varied from 4.1 to 7.8 per cent. Anuria lasting from one to four hours always occurred during the dehydration period. With the administration of water, glucose disappeared from the urine in a few hours. The concentration of glucose in the blood reached from 0.5 to 1 per cent, the latter occurring in an animal that had abstained from water for twenty-four hours preceding the experiment. A fall in concentration to the normal level took place during dehydration, or from two to thirty-six hours after water was administered.

At the end of the dehydration period the urea concentration of the blood was equal to or above the control amount. After the administration of fluid there was a definite rise in three experiments, while in the fourth there was a distinct fall. The excretion of urea in the urine during the diuretic period was typical of that found in water diuresis, lowered concentration with increased total output. When the blood urea did show a rise after the diuresis, a sufficient intake of water soon resulted in a normal

excretion. This excretion was delayed in one experiment owing to definite renal insufficiency.

The excretion of chlorids in the urine was similar to that of urea. There was an associated rise in plasma chlorids of from 9 to 15 per cent, which was directly related to the rise in hemoglobin concentration. Both chlorid and hemoglobin concentration fell to normal on water administration.

The method of production, the rapidity and extent of dehydration caused changes in metabolism, which appear to be chiefly the result of a lack of available water. When water was administered subsequently, a restitution of normal conditions in the organism resulted.

Plethysmographic and pneumographic observations made in hypnosis. G. A. TALBERT, F. L. READY and F. W. KUHLMAN.

Plethysmographic tracings of the arm and respiratory tracings by means of a pneumograph were recorded simultaneously on the same drum to mark the fluctuations produced by suggestion. Control observations were made on the subject while awake of the same suggestions which were to be made while in state of hypnosis. As discovered by others there are constant fluctuations in the volume of the arm without suggestions from the hypnotist, doubtless due to the variable external stimuli. There are marked and predictable changes that follow certain suggestions. The suggestion of cold invariably produces constriction of the arm; likewise, when a subject is held in suspense of a sharp sound to occur at an unexpected moment. Following the sound dilatation is produced. The suggestion of comfort following a discomforting suggestion invariably produces dilatation. Suggestion of heat is not so predictable. If it follows cold, one may expect dilatation. If it follows comfort there will either be constriction or no change. At the beginning of hypnosis there is as a rule a constriction at first followed shortly by dilatation. A final constriction recorded by Walden was not observed by us. Usually a suggestion of awakening produces a constriction but there is a gradual dilatation after awakening.

As suggestions are made one can expect changes in respiration, especially if the subject is laboring under a mental or physical ordeal. These changes are never predictable, even when the same suggestions are repeated at different intervals during the hypnosis.

Proof that anesthetics produce a parallel decrease in the catalase content and oxygen consumption of unicellular organisms. W. E. BURGE.

Chloroform, ether, nitrous oxide and ethylene were the anesthetics used. The unicellular organisms were paramecium (caudatum) and colpoda (Sp.?). A nitrous oxide-oxygen mixture, as well as an ethylene-oxygen mixture, was bubbled through the liquid containing the organisms, at the rate of a bubble every few seconds. Air was bubbled through chloroform, as well as ether, at a slow rate and in turn this chloroform-air mixture and ether-air mixture were passed through the liquid containing the organisms. Catalase determinations were made by adding 0.05 cc. of the organisms to 15 cc. of neutral hydrogen peroxide, and the amount of oxygen liberated in twenty minutes was taken as a measure of the catalase content. The oxygen consumption was determined according

to the Rideal-Stewart modification of the Winkler method. (The determinations of oxygen consumption were made by J. M. Leichsenring.)

Chloroform produced a greater decrease in both the catalase content and the oxygen consumption of paramecium than did ether in keeping with the fact that chloroform was the more effective in anesthetizing the organisms. Colpoda was much more easily anesthetized than paramecium, and the catalase content of this organism is much lower than that of paramecium. Chloroform and ether anesthetize paramecium and colpoda; chloroform being the more effective. Both of these gases produce a parallel decrease in the catalase content and oxygen consumption, but chloroform produces the greater decrease.

Paramecium is much richer in catalase than colpoda. Neither nitrous oxide nor ethylene will anesthetize paramecium, but will produce a slight anesthesia in colpoda. Neither of these gases produces a decrease in catalase or oxygen consumption in paramecium, whereas they produce a slight decrease in colpoda.

The metabolism of cystine. G. J. Shiple, A. R. Rose and C. P. Sherwin.

A number of compounds of cystine and cysteine were prepared in which the sulphydril group or the amino group was "blocked" by means of an organic radical while in some cases both these groups were blocked and in one instance also the carboxyl group. The compounds were fed or injected into rabbits in amounts ranging from 200 to 350 mgm. of sulphur. All the compounds of cystine were oxidized to a considerable extent while those in which both the amino group and sulphydril group of cysteine were blocked were not at all oxidized. This shows that the sulphydril group as well as the amino group is a point at which cystine or cysteine may be attacked by catabolic processes while the carboxyl group has no importance from this standpoint. Evidence was obtained which shows that cystine is readily converted into cysteine and vice versa, as cysteine compounds were isolated from the urine after feeding cystine derivatives and cystine derivatives after feeding cysteine compounds.

Bilirubin formation after total removal of the liver. Frank C. Mann, Jesse L. Bollman and Thomas B. Magath.

Following hepatectomy in the dog there is a gradual accumulation of a yellow pigment in the urine, plasma and fat. In animals which survive for twenty hours or more there is a definite yellow coloration of the mucous membranes and the sclera. The urine, plasma and fat give positive tests for bilirubin, after a period of from four to six hours, and show definite progressive increase in bilirubin content as long as the animal survives. (Gmellin, Adler, Hertzfeld, Rosenberg, Huppert-Cole, and others. The Van den Bergh is at first indirect and becomes biphasic about twelve hours after hepatectomy.)

At corresponding times after operation, the amount of pigment present in the plasma is almost the same as that following extirpation of the gall bladder and ligation of the common duct. That the bilirubin is not dependent on reabsorption from the gastro-intestinal tract, or on any of the abdominal viscera, is shown by its appearance in approximately similar amounts after removal of the liver and the entire abdominal viscera. Extravasated blood in the body cavities or tissues is not a factor in these

experiments, as bilirubin was present in several animals in which no trace of hemorrhage could be found at necropsy.

Injections of laked blood cells in the hepatectomized animal cause a marked increase in the bilirubin content of the plasma. Bilirubin is formed at a rather definite rate without the intervention of the liver, spleen or other abdominal viscera. It would seem that the liver is not essential for the formation of bilirubin, and perhaps should be considered mainly as an excretory organ so far as bilirubin is concerned.

Muscle glycogen after total removal of the liver. JESSE L. BOLLMAN, THOMAS B. MAGATH and FRANK C. MANN.

In hepatectomized dogs there is a marked decrease in the amount of glycogen in the muscles, co-existent with the decrease in blood sugar. The symptoms which appear with a definite hypoglycemic level (0.035 to 0.04 per cent), bear no relation to the absolute amount of glycogen in the muscle, and appear while there is still sufficient glycogen to bring the blood sugar to normal if it is released. Following glucose injections these hypoglycemic animals immediately recovered from symptoms, and if administration of glucose was continued for some time, there was reformation of muscle glycogen.

Injections of other substances (amino-acids, levulose, galactose, sucrose, lactose, inulin, alcohol, glycerol, lactic acid, acetic acid, pyruvic acid), produced no effect on the symptoms, and the animals eventually died unless glucose, glycogen, maltose, dextrose or mannose was given.

Glycogen can be synthesized in the muscle from glucose in the absence of the liver, but in the dog the liver seems essential for the conversion of amino-acids, sucrose, lactose, inulin, galactose, glycerol, lactic acid, pyruvic acid, and so forth, into glucose and glycogen, if these substances are thus transformed in the intact animal.

The effects of insulin in some of the lower vertebrates. FRANK C. MANN JESSE L. BOLLMAN and THOMAS B. MAGATH.

Previous work from this laboratory has shown that following hepatectomy in the lower vertebrates there was always a marked decrease in blood sugar, and that injections of glucose were without effect on the hypoglycemic animals. Since in the higher vertebrates such marked beneficial action followed injection of glucose, we deemed it advisable to test the action of insulin and of glucose on the hypoglycemia following the administration of insulin.

In the gar pike (*Lepisosteus platostomus*), turtle (*Graptemys geographicus*) and frog (*Rana pipens*), the injection of insulin in amounts that would produce marked effects in the common laboratory animals, produced no measurable effect. Intravenous injection of large amounts of insulin produced no measurable effect over a period of from six to eight hours. A marked hypoglycemia was obtained in from twenty-four to thirty-six hours, but the maximal effect was not obtained until from sixty to ninety-six hours after injection.

Injections of glucose or glycogen were without effect in the prevention of the symptoms in the hypoglycemic animals, which is in accord with our findings in the hypoglycemia following hepatectomy.

The changes in the volume of the spleen. ESTES H. HARGIS and FRANK C. MANN.

A plethysmograph was developed which could be applied with sterile technic on the spleens of dogs, allowing it to heal in position. The animals were trained to lie quietly and thus permit observations on the changes in volume of the organ to be made over successive periods of time, and following procedures impossible in acute experiments. We wish to emphasize the following results: *a*, the spleen is extremely sensitive, as judged by changes in volume, to reflex disturbances due to fright and other emotional states; *b*, the spleen undergoes definite spontaneous changes in volume which are modified by fasting and various foodstuffs; and *c*, the action of drugs is the same as in acute experiments.

Comparison of the polarimeter and copper reduction values of glucose solutions. H. L. WHITE, introduced by JOSEPH ERLANGER.

The effect on the optical activity and copper reduction power of dextrose solutions of incubation with insulin and liver extract in M/10 phosphate solution was investigated. Ten cubic centimeters of 11.2 per cent pure glucose solution (Bureau of Standards sample) were added to about 40 cc. of 10 per cent liver extract in M/10 phosphate solution in each of four 100 cc. volumetric flasks. The liver extract was made by shaking 10 grams of fresh hashed beef liver for ten minutes in 100 cc. M/10 Na_2HPO_4, straining through muslin and bringing to pH 7.4 by addition of syrupy H_3PO_4. To two of the flasks 20 units of insulin were added; the other two flasks served as controls The contents of one insulin flask and one control flask were precipitated immediately with tungstic acid, made up to volume and filtered; the filtrates were read in the polarimeter and their copper values determined by the Shaffer-Hartman method. Perfectly clear filtrates were obtained. The other insulin flask and control stood for four hours at 37°, their contents were then precipitated and the polarimeter reading and copper values of the filtrates obtained. The theoretical glucose content of filtrates is 1.12 per cent, i.e., 11.2 per cent diluted 1 to 10.

	POLARIMETER READING	PER CENT GLUCOSE POLARIMETER	PER CENT GLUCOSE COPPER
Control			
1	0.06	1.00	1.12
2	0.07	1.01	1.12
Insulin			
1′	0.07	1.01	1.10
2′	0.08	1.02	1.11

189.4 mm. tube. Zero reading 359.06°. 1 and 1′ precipitated immediately, 2 and 2′ after standing 4 hours.

It will be seen that neither the copper value nor the optical activity of glucose solution is changed in the presence of insulin and liver extract. The work was repeated, using a glucose solution of one-fifth the strength of the above with the same negative results. The results of Winter and Smith (Proc. Physiol. Soc., Journ. Physiol., 1922, lvii, p. xiii) which they interpret as evidence of the formation of γ glucose from α-β glucose in the presence of insulin and liver extract, are not confirmed. No explanation is offered for the consistently lower figures obtained with the polarimeter than by copper reduction.

The effects of intravenous injections of glucose at a constant rate on blood sugar and blood volume. ERRETT C. ALBRITTON, introduced by R. G. HOSKINS.

Glucose was administered intravenously to dogs at the constant rate of 0.7 gram per kilo per hour for two and a half hours, and changes in concentration of blood sugar and of hemoglobin were followed. Blood samples were taken before and at approximately 20, 30, 40, 50, 62, 75, 111 and 150 minute intervals during the injection. About thirty curves of blood sugar and twenty-five of hemoglobin changes were obtained.

After a sharp rise in the first 20 minutes, the curves usually formed an irregular plateau at an average of 50 mgm. per 100 cc. above the initial level. In only two experiments did the curve fall as low as the initial level. In others a fall with secondary rise or a gradual rise or irregular oscillations occurred during the plateau period. Two curves continued to rise without pleateau formation. Several experiments were performed in which blood was taken every 3 to 6 minutes. In most of these frequent and sometimes wide oscillations occurred.

The curves of hemoglobin concentration usually fell in the first twenty minutes or more, and remained below the initial level, often running a quite irregular course, sometimes rising temporarily even above the initial level, and rarely running for the major part of their course above this level.

On the assumption that changes in sugar and hemoglobin concentrations found in jugular blood were reflections of similar changes in the total blood, and that the blood volume at the beginning of each experiment was approximately 92 cc. per kilo, the quantity of circulating sugar per kilo was calculated and curves plotted. These "quantity" curves followed their respective concentration curves fairly closely, but usually showed still greater irregularities in course. Since in these experiments the rate of intake was constant, changes in the quantity curve were assumed to be due to changes in the rate of disappearance of sugar as such from the blood, and a curve of the disappearance rate was plotted. Within a period of 20 to 30 minutes the disappearance rate came into approximate equilibrium with the rate of intake, but often with wide and frequent oscillations above and below this latter rate. There was no indication in these curves of a preliminary latent period with an abrupt rise of the disappearance rate at the end of the 20 to 30 minutes, due to the sudden operation of an assimilation facilitating factor, as proposed by Maclean and de Wesselow.

Gases of the swim-bladder of the California phosphorescent fish, Porichthys notatus. C. W. GREENE.

The gaseous content of the swim-bladder has been studied in the phosphorescent fish, Porichthys notatus, which inhabits the coastal waters of California and Monterey Bay. The swim-bladder serves primarily as a vocal organ. It is embryologically a pharyngeal evagination but is entirely separated in the early stages of development. If the gas is emptied from the cavity, it is promptly refilled within four hours or less. The bladder has the usual reta mirabile and so-called oxygen gland. The fact is emphasized that all analyses show a very high per cent of oxygen, a supplementary amount of nitrogen, and small but variable quantities

of carbon dioxide. The oxygen content varies through a wide range, 26.9 to 88.2 per cent. In most specimens the percentage varies between 50 and 88.

The per cent of first samples is as high or higher than succeeding samples. This is not in agreement with Bohr's findings that first samples contained less oxygen than atmospheric air, while refilling samples contained greater percentages of oxygen.

When the air-bladder is emptied and then refilled with atmospheric air under greater than the normal atmospheric pressure, the composition of this air is changed by a relative increase in oxygen and decrease in nitrogen. This suggests that the nitrogen can be removed from the bladder as well as, and probably at the same time that oxygen is added to the air-bladder.

One rare instance was observed in which the gas separation resulted in an over-inflation of the air-bladder to over 100 cc., a degree that left the fish floating helplessly on the surface of the water. This gas contained over 82 per cent of oxygen, a proportion that cannot be accounted for on purely physical diffusion pressures.

Evidence is not now offered on the nerve control of the so-called oxygen secretory mechanism.

The excretion of certain dyes and other substances in the frog's kidney and its bearing upon the theory of renal secretion. R. N. BIETER and A. D. HIRSCHFELDER.

Direct observation of the frog's kidney by Richards' method shows that sodium benzoate increases the number of glomeruli with active circulation while sodium hippurate does not. This confirms the theory of Kingsbury and Swanson and of H. B. Lewis, that hippuric acid is formed in the kidney.

Phenolsulphonephthalein, sodium indigosulphonate and trypanblue are excreted in the glomeruli and can be seen in the space between the capillary loops and the capsule, and somewhat later in the lumina of the tubules but not in the cells of the tubules or in the interstitial lymph channels. This has also been shown independently of us by Richards and Wearns. The dyes are excreted in the glomeruli only. If the branches of the renal arteries to the lower pole of the kidney are cauterized, circulation through the glomeruli stops in this region and no dye is excreted there, even though an active circulation through the vessels supplying the tubules persists. Wherever glomerular circulation is intact and the dye is excreted in the tubules it is many times deeper in color than in the glomeruli in spite of the fact that the lumina of the tubules are about one-fourth the diameter of the glomerular capsular spaces. Therefore the urine must be concentrated to one-tenth or one-twentieth its original volume and the process of concentration occurs at least in the first part of the tubules. These observations furnish the first direct objective proof of the theory that the urine is concentrated in tubules by the reabsorption of water.

The peripheral water loss in rabbits as a factor in heat regulation. ROBERT W. KEETON.

A normal resting rabbit exposed to an atmosphere, the temperature of which by dry bulb is 104°F. and by wet bulb 79°F. or lower, maintained its rectal temperature of 103°F. over an experimental period of two to four hours. If the wet bulb temperature was increased to 82°F. and the dry

bulb maintained at 104°F. the rabbit's temperature rose steadily. The height attained was determined by the duration of the experiment. Under these experimental conditions the heat produced by the rabbit must of necessity all be lost in the form of latent heat of vaporization. This work is simply an extension to the rabbit of Haldane's experiments on man.

A rabbit placed in a container in such a way as to prevent all peripheral heat loss, so that the sole and only method of heat dissipation was through the respiratory surface, showed a steady and progressive rise of temperature. In four and one-half hours the rabbit's temperature reached 111.5°F. Analysis of expired air from animals with constant rectal temperatures under somewhat similar conditions showed a heat production ranging from 5.7°C. to 10°C. per hour. Even if the rabbit breathed an absolutely dry air at 32° he still could not eliminate this quantity of heat through the respiratory surface.

If therefore it is possible for a rabbit to eliminate all of his heat through vaporization of water, and if it is impossible for him to vaporize all of this water from the respiratory surface, then it follows that the periphery must be a very important surface for the elimination of water. In the absence of sweat glands this water loss must be attributed to some form of physical transudation. The ears can be conceived of as excellent radiators through which evaporation may proceed maximally. The significance of this observation lies in its extension to man.

Growth and metabolic changes in maize seedlings treated with insulin or glucokinin. M. M. Ellis, introduced by C. W. Greene.

The reactions of maize seedlings still dependent upon the endosperm for food, in root growth, top growth and pigment formation to various solutions of insulin and glucokinin, as previously reported by Ellis and Eyster (Science, lviii, 541, 1923), have been verified by additional series. Following the differences in the action of insulin and glucokinin solutions on seedlings having their root systems intact, as compared with the action of similar solutions on seedlings from which the tips of the primary roots had been removed, an effort was made to separate the fractions producing these different responses. By the dialysis of glucokinin prepared by Collip's method from onion tops or corn plants through a Marriott's celloidin tube two fractions have been obtained. The residue remaining in the celloidin tube contains the substance or substances which increase growth and the dialyzate contains those which repress growth. When seedlings were grown in strong solutions of the dialyzate from onion glucokinin drops of brown, sticky fluid, rich in sugar, were exuded at the ends of the leaf-tips from the hydathode, after three or four days. Following this sweating of sugar the leaves wilted and died. Disturbances in the starch storage in the leaves of seedlings grown in the dialyzate have also been noted. The dialysis of crude insulin has given fractions comparable to those obtained from glucokinin, although the reactions produced in the seedlings have not been so marked.

The effects of thyroid feeding and thyroxin injection on the basal metabolic rate, temperature and body weight. Margarete M. Kunde, introduced by A. J. Carlson.

I. *Thyroid feeding.* In single doses, 4 grams to 150 grams, the basal metabolic rate is always increased for doses of 50 grams or more Four to

10 grams produce negative, or only slightly positive, results on the basal metabolic rate. Temperature for small doses is negative; it is increased with large doses.

With daily doses of 4 grams per day, the basal metabolic rate is increased from 30 to 40 per cent after four to five days. There is a return to normal about three days after discontinuing the thyroid feeding. There is no effect on body weight or temperature. The pulse rate is increased.

II. *Thyroxin injection.* A single dose of 3.5 to 20 mgm. may slightly increase the basal metabolic rate on the second day after injection. This increase is accompanied by a rise in body temperature and an increase in pulse rate.

Daily doses of 2 mgm. increase the basal metabolic rate 30–50 per cent with a marked increase in temperature and pulse rate. Body weight remains constant.

A quantitative comparison of the toxicity of the alkamine esters of aromatic acids used as local anesthetics. CHARLES W. HOOPER and ELSA BECKER.

The method used was a modification of the official arsphenamine toxicity technique in which healthy, non-pregnant albino rats weighing between 100 and 150 grams are employed. The rats were obtained from one source

TABLE 1

Comparative variation in the toxicity results obtained in the albino rat and cat after intravenous injection of the alkaline esters of the aromatic acids used as local anesthetics

	ALBINO RATS		CATS	
	Maximal tolerated and minimal lethal doses	Strength of solution	Fatal dose	Strength of solution
	mgm. per kgm.	*per cent*	*mgm. per kgm.*	*per cent*
Butyn	7.5–10	2	15*	1
Alypin	10–15	2	10†	5–10
Cocain	12.5	2	15†	5–10
Tropacocain	15–20	2	18–22†	10
Apothesine	20	2	20†	5–10
Beta-eucain	15–25	2	10–12.5†	5
Stovaine	25–30	2	25–30†	10
Novocain	45–55	2	40–45†	5–20

* Fatal dose obtained by Prof. Robert A. Hatcher. See Bonar and Sollman: Journ. Pharm. Exper. Therap., 1922, xviii, 475.

† Fatal doses obtained by Prof. Cary Eggleston and Prof. Robert A. Hatcher. Journ. Pharm. Exper. Therap., 1919, xiii, 444.

and kept on a constant diet for at least two weeks before the tests. They were fasted for from sixteen to twenty hours immediately before the injection of the drugs, were weighed and the doses administered. Water was supplied during the fast period. A 2 per cent solution of the drug in distilled water was injected into the saphenous vein at the rate of twelve to fifteen seconds for every 0.1 cc. At least five rats were injected at each dose. The animals were kept under observation for twenty-four hours, during which time they were fed the routine diet. The minimal dose at which any of a series of animals died is considered the maximal tolerated

dose. The dose at which the majority of a series died is considered the the minimal lethal dose.

It will be noted that the results obtained by the two methods are quite comparable and that there is very little variation in the maximal toxicity for the two species when the drugs are injected intravenously. The difference in the individual susceptibility of the animals is slight. The variation between the maximal tolerated dose and the minimal lethal dose does not exceed ten milligrams in any of the experiments. The results indicate that the method from an experimental viewpoint may be considered quantitative.

The influence of sodium bicarbonate and calcium carbonate on the secretion and composition of gastric juice. THEODORE E. BOYD.

Pavlov pouch dogs were used to test the view that the alkalies depress gastric secretion. Each animal received sodium bicarbonate at intervals through the day. The daily dosage was gradually increased up to 6 grams per kilo weight. In some doses the bicarbonate was combined with an equal amount of calcium carbonate, in an attempt to control diarrhea. The period of dosage was from thirty to seventy-five days and controls were run for several days before and after.

The average secretory response to feeding was somewhat greater during period of alkali treatment than during the first control, although secretion was depressed after the maximum doses. There was a further increase, for several days, after the withdrawal of the alkali. Depression following the large doses was accompanied by evidence of gastro-intestinal irritation, diarrhea or vomiting.

The average of more than fifty experiments with single doses of sodium bicarbonate every fourth day, with controls in the intervals, showed that at least one gram per kilo body weight, at one time after feeding, was required to appreciably depress secretion. Smaller doses were indifferent, or stimulating. The depressing effect was somewhat more definite when bicarbonate was given with water alone.

This depression may be a nervous inhibition, due to thirst, alkalosis and nausea. But it is not due to thirst alone, for it is manifested almost as readily when the water intake is unlimited. Chemical changes in the blood may also be involved since the plasma chlorides somewhat diminished after large doses of bicarbonate.

Experiments with calcium carbonate showed on the average an increase in secretion up to 3 grams per kilo at one time. The effect was not constant and showed no definite relation to the dosage.

Conditions effecting the separation and migration of plant cells. WILLIAM H. CHAMBERS.

It has been shown by Robbins and confirmed by Kotte that the excised root tips of young seedlings will grow in vitro to form normal differentiated root tissue with secondary and tertiary branches. Large fragments of embryonic tissue, 1 to 10 mm. in length, were used for these cultures. In cultures of plant tissue, however, a separation and migration of the individual cells such as is found in the cultures of tissue from the higher animals has not been reported. In recent experiments in which very small fragments, less than 1 mm. long, of embryonic tissue were grown in hanging

drop culture for microscopic study the migration of individual cells has been observed. This separation and migration of the cells from the fragment is similar to that seen in animal cells.

Squash seeds were sterilized in chloramine-T and germinated in 1 per cent agar. When the radicle had grown to 20 or 30 mm. fragments of embryonal tissue were cut from the root tips and planted in a nutrient medium of salts, peptone, dextrose and agar. The reaction of the medium was buffered with potassium phosphates to pH 5.6, which was the pH of a ground cotyledon extract and a ground squash seedling extract.

This migration of the individual cells, like that in the animal tissue cultures, is greatest on the surface of the medium, although it also occurs through the center of the drop. On the upper surface of the agar, next to the cover glass, migration has been observed to a distance of 1.7 mm. from the tissue. On the lower surface a viscid film is seen diffusing from the tissue with the cells distributed more or less evenly through it. The cells are not merely floating free in a fluid, but are held immobile against shaking. A similar surface film has been reported in cultures of young embryonic animal tissue and cancer tissue, but not in cultures of older embryonic or adult tissue in a salt solution medium. The migrating cells are not the dead, plasmolyzed outer cells of the root cap but are viable, round and ovoid cells. After migration has ceased, some of the isolated cells become plasmolyzed, others remain viable for many days.

While the migration of the plant cells resembles that of the cells of the higher animals, growth in the cultures of these small fragments is different in so far as it has been observed. The animal cells grow in mass without forming a definite structure. Mitosis has been observed in isolated cells. In plant cultures, growth has always taken place in the fragment to form organized and differentiated root tissue. No mitoses have been seen in the cells separated from the fragment.

The data show a definite relationship between the length of the excised root tip and the growth and migration from it. Typical migration was found only in fragments under 1 mm. in length (including the root cap). The best migration was from tips between 0.5 to 0.8 mm. long. The growth was measured by increase in length of the tissue fragment. The average growth of the fragments increased directly with the size of the fragment planted. The average growth from 0.5 to 0.6 mm. fragments was 0.27 mm., from 0.6 to 0.7 mm. was 0.65 mm., and from 0.7 to 0.8 mm. was 2.27 mm.

These data suggest that plant cells and animal cells will agree in many of their fundamental physical reactions.

Some measurements of the polarization of metals used as electrodes. M. DRESBACH and HELEN R. HOSMER.

To study the extent to which the various metals used as electrodes polarize under various conditions, a special method has been developed, taking advantage of the three-electrode amplifying tube, which makes it possible to study on a practically open circuit the voltage changes involved. This is accomplished by connecting in turn to the grid-filament circuit known potential differences and those to be studied, while recording by the string galvanometer the resultant changes produced in the output, or plate-filament, circuit. This procedure makes it practicable to measure

the initial values and rate of decline of polarization produced by impressed potential differences of a few millivolts.

A potential of 30 millivolts was chosen for the initial E. M. F. to be applied. This is about ten times the maximal voltage met with in electrocardiography. The different metals examined had a surface area of about 36 sq. cm. for each plate of a pair. They were all immersed in pure 25 per cent NaCl solution and polarized for the same length of time (5 to 30 seconds). On the photographic records of the galvanometer deflection we measure the polarization voltage, the method being accurate at present to 0.5 m.v. The data obtained enable us to arrange the metals studied in the following order, placing at the head of the list the one polarizing the least under the experimental conditions; the amalgamated zinc—zinc sulfate electrode; the silver—silver chloride electrode; lead; zinc; tin and uncoated silver.

Reducing the surface area of the coated silver electrode to about one-tenth of the original area (36 sq. cm.) made very little difference in the degree of polarization. With Pb, Zn and Sn, however, polarization is much increased. Also, the silver electrode must be kept in the dry state while not in use. It will then remain in good condition for a very long time.

The effect of intravenously injected calcium lactate. JULIUS BLUMENSTOCK, introduced by A. B. LUCKHARDT.

We record the following observations. A thyroparathyroidectomized dog showed signs of depression a month after double ovariotomy. To improve her condition 3.6 grams calcium lactate diluted in 400 cc. of Ringer's solution were injected intravenously. The immediate effect was an acute distress and anuria of at least six hours' duration. When the secretion of urine was resumed, it was greatly reduced in amount and contained 18 per cent albumen. The albuminuria decreased in severity, but at death a month after it was present to the extent of ½ to 1 per cent. Post-mortem examination showed a partial obstruction of the duodenum arising from the right ovarian ligament, and a marked destruction of the kidneys, both glomeruli and tubules being involved.

In the further study of intravenously injected calcium lactate, the following method was employed. Four hours after a meat meal, normal unanesthetized dogs were injected with 1 cc. (6 mgm.) phenolsulphonephthalein solution and with 25 cc. per kilo body weight of Ringer's solution in which were dissolved 1 to 5 grams of calcium lactate. Control experiments without the injection of calcium lactate were performed. The following effects were observed:

1. During and for a variable period of time after the injection, the animals showed signs of acute distress, manifested by vomiting, shivering, convulsions, hyperpnea and marked salivation.

2. In some animals there was an intense blanching of the mucous membrane of the mouth and vulva, undoubtedly symptomatic of a vasoconstriction of all peripheral vessels. This effect persisted often for two hours.

3. In all cases there was a diminished secretion of urine as compared with controls; in some there was a complete anuria of an hour's duration. In no instance was the excretion of phenolsulphonephthalein during a two-hour period appreciably diminished.

4. In every case the final result of the injection of calcium lactate was an albuminuria, which was proportional to the amount injected. But even with massive doses we were unable to produce the degree of nephritis noted in the first experiment.

5. The urine was collected continuously from a catheter into the bladder. The reaction of the urine would vary from acid to alkaline 3 to 5 times during a fifteen-minute period, and the changes were in no manner related to the rate of flow.

The action of the vagus as a conservator in bulbar anemia. HELEN C. COOMBS.

I have reëxamined the effects of occlusion of the head arteries of the cat upon the anemic blood pressure curve and on the heart rate under the methods of Stewart, Güthrie, Burns and Pike, 1906, with special reference to the effects of division of the vagi before and during occlusion. If vagotomy is done before occlusion, the anemic curve lacks the vagus dip and often the rise in blood pressure is not so high. If the vagi are divided about the middle of an occlusion period, pressure quickly rises from 10 to 30 mm., and in the subsequent occlusions attains the greater height. Moreover the heart rate, which undergoes typical changes during an occlusion with vagi intact, is unchanged after the vagi are divided. Table of typical data:

	HEART RATE BEFORE OCCLUSION	20 SECONDS AFTER OCCLUSION	130 SECONDS AFTER OCCLUSION	20 SECONDS AFTER RELEASE	LENGTH OF OCCLUSION PERIOD
Second occlusion vagi intact.....	198	165	246	174	170 sec.
Sixth occlusion vagi intact.....	168	146	210	186	180 sec.
Tenth occlusion vagi divided.....	216	216	50 sec. after 216	216	75 sec.

These data are in agreement with Hooker's view that the heart attains its maximum rate after section of both vagi.

A normal cat will stand from 10 to 20 occlusions of the head arteries, under the usual conditions. If the vagi are divided, the number of occlusions before the medulla is completely exhausted is diminished and the length of each anemic rise is reduced.

In one experiment the first two occlusions occupied respectively 240 and 270 seconds before the beginning of the fall in pressure to spinal level. The vagi were then divided. The length of the third occlusion period on the same basis was only 120 seconds.

The results after atropine were identical with vagotomy which argues for exclusion of afferent vagal components.

It would seem that the vagus functions during the period of stress imposed by ligation of the head arteries as a conservator, by tending to keep the arterial pressure down by regulating the heart rate. Its sparing effect on the medulla is shown by the greater duration of the periods of bulbar anemia when the vagi are intact.

Organisms of the enteritidis paratyphoid B group as causative factors in varying blood sugar content of rabbits. MAUD L. MENTEN and HELEN M. MANNING (by invitation).

Coincident with the isolation of B. enteritidis and B. paratyphosus B of animal origin from organs at autopsy of guinea pigs and rabbits, characteristic lesions were found in various organs. Those in the pancreas occurred mainly in the islets of Langerhans, the cells of which showed either hydropic degeneration or pyknotic nuclei. Both the organisms occur in the intestinal tract of various rodents, and rabbits inoculated with either of them show gradual increase in blood sugar to 200 mgm. per 100 cc. blood with a final rise to 235 to 250 mgm. Immediately previous to death, hypoglycemia (40 to 50 mgm.) occurs.

Immunization against B. paratyphosus B was not successful, as agglutination was not obtained in dilutions above 1:700, and no reduction was observed in blood sugar values. Charts indicate a fall to normal in two groups of rabbits after immunization with B. enteritidis in which agglutination of 1:2000 and 1:8000 dilution was obtained, but these sugar values did not remain permanent. This immunization, therefore, while it protects against B. enteritidis, offers little protection against B. paratyphosus. The reduction in blood sugar was probably mainly occasioned by rise in temperature, as we have observed that during warm weather sugar values lie between 80 and 115 mgm. while they rise under unfavorable conditions, such as cold weather, bad housing, infections, etc., which lower the animal's resistance. This increase in blood sugar in the rabbit we believe to be due to invasion of the blood stream and organs by these bacteria from the intestinal tract. Since pathological changes occur in pancreas, liver and kidney, it is probable that each plays a part in altering percentage of blood sugar.

The effect of habituation on the rotation-nystagmus as compared with the after-nystagmus in the rabbit. S. S. MAXWELL.

It had been previously shown that in rabbits the amount of after-nystagmus following rotation decreases with habituation. Animals which showed a pronounced after-nystagmus on the first day of rotation were given 200 rotations daily in alternative sets of 10 turns to the right and 10 turns to the left. In the course of a few days, or a few weeks at most, the after-nystagmus became negligible in amount or entirely disappeared. In those experiments the duration of the after-nystagmus was measured with a stop watch and the number of eye movements were counted and recorded. It was noticed that when the after-nystagmus had disappeared the rotation-nystagmus appeared normal.

The present investigation has been undertaken to discover what relation, if any, exists between the rotation-nystagmus and the after-nystagmus. The animal's head was fixed in a Czermak holder. The nystagmus was recorded graphically by the method of Delabarre. The moment of stopping was marked by an electric signal and time intervals by a Jacquet clock. All the animals used gave normal responses to the caloric tests at the close of the experiment.

1. There is a reduction both of rotation-nystagmus and the after-nystagmus but in very different proportions. At a time when the after-nystagmus had practically disappeared the rotation-nystagmus had not

usually been reduced as much as 50 per cent. Indeed it is doubtful if the rotation nystagmus would ever disappear under the conditions of these experiments.

As a specimen case we may take the record of rabbit 1.

DATE	ROTATION-NYSTAGMUS	AFTER-NYSTAGMUS
9/24	93	19
9/25	69	10
9/26	71	6
9/27	63	5
(3 day intermission)		
10/1	76	1

2. In rabbits which have not been subjected to rotation the individual differences in the after-nystagmus are very much greater than in the rotation nystagmus. Thus on the first day of rotation these data were observed.

RABBIT	ROTATION-NYSTAGMUS	AFTER-NYSTAGMUS
1	93	19
2	92	17
3	88	28
4	93	3
5	86	8

Our results show that the amount or duration of after-nystagmus following rotation is no reliable indication of the functional activity of the equilibrial apparatus.

The effect of light and of darkness on the sign of the heliotropism of the water-boatman, Corisa. S. S. MAXWELL.

Many examples are known of the effect of light differences on the character of the heliotropic reactions of animals. In most of the instances the effect of strong light is to decrease the positive reaction or to bring about a negative reaction. On the contrary in weak light negative organisms may become positive and in some cases animals which are indifferent in ordinary daylight become strongly positive after having been kept for a time in darkness. Very few instances appear in the literature of animals which are positive in the light and which become negative after having been kept in the dark.

For the above reason it appeared desirable to put on record some uncompleted observations on *Corisa*. Specimens when collected and brought to the laboratory manifested an intense positive heliotropism. When a strong electric light was turned on in the dusk of the evening they swam rapidly to the side next the light and a great number of them emerged from the water and flew to the electric light bulb. Some dishes of the specimens were left in the laboratory, other dishes were put in the dark-room. At 10 o'clock the following morning, all the specimens in the laboratory were strongly positive in their reaction to light. On the contrary those in the dark-room, in which they had been kept approximately sixteen hours, were strongly negative when first exposed to the light. After four or five minutes' exposure they became indifferent and then a short time later were all distinctly positive. This observation was repeated a number of times with very striking results.

Endocrine and associated disturbances induced in the rat by operations upon the pituitary and the effect of a replacement therapy upon certain of these disturbances. PHILIP E. SMITH and JAMES B. GRAESER, introduced by S. S. MAXWELL.

The injection of chromic acid into the hypophysis of the rat has induced a typical adiposogenital syndrome in seven cases. The adiposity may be extreme as shown by a male, aged five months, operated upon at the age of two months, which now weighs 445 grams as compared to 380 grams for its litter-mate brother, although through inhibition in its skeletal growth it is 7.5 cm. less than the latter in total length. The females display no oestrous cycle. Two of these have been autopsied. Their ovaries were greatly reduced in size, contained no recent corpora, and but few large follicles all of which together with many of the small and medium sized ones were undergoing atresia. The thyroid glands of these animals were approximately one half the size of those of their normal litter mate sisters. The follicular epithelium of these glands was greatly flattened in contrast to the normal cuboidal type of the controls. The adrenal glands were even more strikingly modified averaging but one-fifth the weight of these glands in the controls. This reduction in size was due largely to a decrease in the cortical tissue.

A study of serial sections through the hypophyseal region revealed the presence of a considerable amount of neural and intermediate and a small amount of anterior lobe tissue, though in one animal no typically staining anterior lobe cells were found. No definite injury to the hypothalamus was evident. Several control operations without injury to the pituitary region caused no interruption in oestrus or other abnormalities.

A replacement therapy was instituted on two animals which had exhibited no oestrous cycle since the operation, although abnormal adiposity had not developed. The ovaries obtained from unilateral ovariotomies before treatment was commenced were undersized, contained no recent corpora and no large follicles. In both cases oestrus was induced by the injection of anterior hypophyseal substance. One animal has been autopsied. Its remaining ovary was of normal size, contained many corpora and normal follicles.

The inhibition of the stomach in Necturus. T. L. PATTERSON.

Previous work on the movements of the empty stomach in the Necturus has indicated that the controlling influence of the vago-sympathetics is largely inhibitory. This phenomenon is contrary to that which exists in the stomachs of higher animals, although in the frog and turtle the tenth nerve contains both motor and inhibitory fibers to the stomach with the motor predominating, at least in the frog. The method used consisted of transecting the spinal cord between the first and second cervical vertebra under ether and stomostomy near the angle of the mandible for the introduction of a rubber recording balloon into the stomach. The vagi were isolated dorsally and near their exit from the cranium for a distance of 3 to 5 mm. and lifting ligatures placed under each nerve. These nerves can be isolated without hemorrhage. The incisions were plugged with cotton moistened with normal saline and the animals were placed dorsal side up on a specially constructed animal holder with water troughs, whereby the gills were constantly covered with running water, both from above and below. Respiration and circulation are fairly normally maintained for

from three to five days during which time the effect of the vago-sympathetics can be determined on the stomach.

Stimulation of the vago-sympathetic, even slight traction produced inhibition or stoppage of the movements of the empty stomach. Ligaturing and sectioning of both vago-sympathetics first produced inhibition of the gastric movements due to mechanical stimulation followed in a short time by the return of the gastric contractions in augmented form, the augmentation even greatly exceeding the normal contractions with the nerves intact. These findings tend to show that the fibers contained in the vago-sympathetics to the stomach in Necturus are largely inhibitory. Evidence so far obtained also points to the fact that the vago-sympathetics in Necturus contain both motor and inhibitory fibers to the stomach with the inhibitory type predominating. The predominance of inhibitory fibers is apparently in inverse order to the arrangement in other animals.

The effect of the removal of one kidney on the function and structure of the remaining kidney. T. ADDIS, B. C. MYERS and JEAN OLIVER.

Previous investigators have found that the removal of one kidney has little or no effect on such measurements of function as are commonly used as indices of the work of the kidney. But we have found that the underlying relation between structure and function can be demonstrated when the factor of strain is introduced. In rabbits which were given large amounts of urea and water, unilateral nephrectomy was followed by a marked decrease in the rate of excretion of urea and of water. The closest correlation between structure and function was attained when the ratio $\frac{\text{urea in one hour's urine}}{\text{urea in 100 cc. of blood}}$ was taken as the measure of function. Three months after the removal of one kidney the remaining kidney was found to have increased in weight from the original 50 per cent to 66 per cent of the estimated renal weight if no nephrectomy had been done, while the ratio measurements showed 79 per cent of the original function with both kidneys. But on microscopical examination of the remaining kidney it was found that the hypertrophy was not diffuse but affected particularly the convoluted tubules and the glomeruli, while other parts of the kidney were only slightly if at all enlarged. In these kidneys, therefore, the relation between the mass of the convoluted tubules and glomeruli and the mass of the kidney as a whole was different than that which obtains in the normal kidney. There are anatomical reasons for the belief that the convoluted tubules are especially concerned in the excretion of urea, so that it is possible that the discrepancy between the anatomical and functional measurements may in whole or in part be accounted for by the fact that in this special instance the weight of the whole kidney did not adequately represent the actual amount of urea secreting tissue.

Evidences of electro-positivity in the heart due to vagus stimulation. WALTER E. GARREY.

Vagal "positivity" in the heart as reported first by Gaskell has been questioned by Einthoven and Rademaker, who attribute this electrical change to secondary effects resulting from contraction of muscle in the lungs. The contention of the latter authors is controverted by our experimental results which show that "positive variation," due to vagus stimu-

lation, can be demonstrated *a*, in the heart of selachians which have no lungs; *b*, in the turtle's heart (auricles) removed from body in such a way as to preserve both functioning vagi; *c*, in the excised isolated auricles of the alligator heart when the vagus fibers in the coronary nerve are stimulated. *d*, The changes of electrical potential (positivity) are chiefly homolateral, i.e., in the auricle on the side of the vagus stimulated as shown by differential leads from the two auricles; *e*, these differential homolateral effects are identical with those obtained (*1*), when the heart has been separated by sagittal section or clamp into lateral halves each innervated, by its homolateral vagus, or (*2*), when the auricles are directly faradized, and can be accounted for only by vagus effects localized in the heart; *f*, the changes in potential due to vagus stimulation, whether in the heart in situ or in the excised heart, quantitatively exceed those due to simple arrest, but this excess of positive potential can be demonstrated only in those parts of the heart in which other evidences of inhibition as contrasted with "arrest" are demonstrable. *g*, Atropin locally applied to the heart eliminates positive variation due to vagus stimulation.

The rôle of the thyroid gland in the growth of the brain and spinal cord from the point of view of phylogenetic evolution. FREDERICK S. HAMMETT.

The growth capacity of the brain of the albino rat of both sexes is reduced by the removal of the thyroid apparatus to a much greater degree than is that of the spinal cord. Moreover, the growth capacity of the spinal cord in thyroidless rats is but slightly affected by sex or age differences at time of extirpation. On the other hand the growth capacity of the brain tends to follow the sex and age differences exhibited by the body as a whole in its response to thyroid deficiency. This greater dependency of the brain upon a functioning thyroid gland for its normal development is interpretable from the point of view of phylogenetic evolution.

The spinal cord has developed through the course of phylogenetic evolution its characteristic structure and composition independent of thyroid influence. On the other hand, the evolution of the brain, and particularly the cerebral hemispheres the growth in weight of which is represented in these studies, has come along later than that of thyroid function. It has, therefore, been associated with the thyroid influence from its beginnings. Therefore the growth and development of the brain should be more dependent upon thyroid function than that of the spinal cord.

Synergism between sodium cyanide and minimal electrical stimulation of the vagi on the respiratory center. C. D. LEAKE (by invitation), H. S. GASSER and A. S. LOEVENHART.

Dogs were maintained under light ether anesthesia. Both vagi were exposed and connected by shielded electrodes to the secondary circuit of an induction coil, and the injections were made into the external jugular veins. The minute-volume of respired air was found by using a body plethysmograph, sensitive to 2 cc.

The positions of the secondary coil necessary to secure the effects of subminimal vagal stimulation on the respiratory center were determined for each animal, and the amounts of N/100 or N/200 NaCN which would

cause a minimal stimulation of respiration upon intravenous injection were also found. Then the effects of these two procedures occurring simultaneously were determined, and in every case it happened that the respiratory stimulation was considerably greater than for either procedure alone and greater than a simple summation of the two. Neither the duration of vagal stimulation nor the sequence of procedure had any significant effect on the phenomenon.

Sodium cyanide, as is well known, reduces oxidative processes. The physiological response to a reduction of oxygen fixation by the cell under proper conditions is increased activity. The degree of stimulation is determined by *1*, the suddenness with which this reduction is brought about; *2*, the extent of the reduction; and *3*, the condition of the cells. Since minimal vagal stimulation so markedly augments the stimulation of the respiration by sodium cyanide, it would seem at least a possibility that this nervous impulse reduces oxygen fixation in the cells of the respiratory center.

A reversible reaction of the vagi to nicotin. J. E. THOMAS and ALBERT KUNTZ.

Bayliss and Starling (1899) stated that 0.4 to 2.0 cc. of 1 per cent nicotin produces complete and permanent paralysis of the vagus to the small intestine in dogs. In an experimental study of intestinal motility we failed to obtain vagus paralysis with doses of nicotin even a hundred times larger. We have investigated the apparent discrepancy. In twelve dogs, under ether, both vagi were severed in the thorax. The splanchnics also were severed. Intestinal movements were recorded by the balloon method. One per cent and five per cent solutions of nicotin hydrochloride were used.

Stimulation of the peripheral ends of the vagi usually produced the typical results described by Bayliss and Starling, i.e., a primary inhibition followed by an increase in both the tonus and the amplitude of the rhythmic contractions. Small doses of nicotin (0.25 to 1 cc. of 1 per cent solution for 5 to 8 K. dogs) rendered the vagi entirely ineffective as stated by Bayliss and Starling. On continuing the experiment with increasing doses of nicotin this apparent paralysis subsided and again vagus stimulation became effective. Vagus irritability increased with this increase in dosage until a total dose of 0.5 to 1 cc. of the alkaloid per kilo had been administered. At any rate stimulation of the vagi at this stage produced a greater increase in tonus and in amplitude of the contractions than before the injection of nicotin. With still further increases in dosage the effects of vagus stimulation diminished. They subsided completely when the dosage approximated that required to completely paralyze postganglionic sympathetic fibers (2 to 3 cc. of the alkaloid per kilo of body weight).

The primary inhibition characteristic of the effect of vagus stimulation on the small intestine in anesthetized animals without nicotin did not appear in any of our records after giving nicotin.

The mode of action of adrenalin chloride on the respiratory center. L. B. NICE and ALMA J. NEILL.

The action of adrenalin chloride on the respiration of urethanized rabbits, cats and dogs was studied. The respiratory movements were recorded as in previous experiments (Nice, Rock and Courtright, THIS

JOURNAL, 1914, xxxiv, 326). Synchronous records were also made of blood pressure by mercury manometers one from the central end of the carotid artery and another from the peripheral end of the same artery, thus giving simultaneous records of respiration and the blood pressure in the head area and the central region of the body. The adrenalin was introduced in varying strengths either into the peripheral end of a carotid artery or into the central end of an external jugular vein.

Our results indicate that adrenalin affects the respiratory center directly. In general there may be an increase in the depth of respiration with small doses and a decrease in the depth with large doses.

Blood pressure variations in vitamin deficient rats. F. M. BALDWIN and H. B. COOK.

Using a modified recording mercury manometer, direct pressure curves were obtained from the abdominal aorta of anesthetized rats previously fed upon specific diets. The average of twenty-two animals on growing ration was 84 mm. Hg. B-deficient animals had a conspicuously low blood pressure. In 16 cases the average was 34 mm. Hg. Histological study of the heart tissue of these revealed marked hypertrophy of the ventricular walls; multiple foci of ecchymoses and areas of edema of the apex. These findings conform to conditions found by McCarrison in monkeys and Durck in humans, but disagree with Uhlman since he found that administrations of vitamin B extracts lowered the heart action and blood pressure in rabbits and dogs.

A-deficient animals showed constant though slight subnormal pressures. The average of 19 animals was 75 mm. Hg. Histological examination of the hearts of these showed marked atrophy; a segmentation of muscle fibers; slight congestion of the myocardium but no edema and nondilated walls. The apparent proximity of pressure in these to the normals may be reconciled by assuming compensatory tachycardia; increase in minute volume; and a hypertension of the arterial system due to vasomotor disturbances; or possibly changes which alter the viscosity of the blood.

Histological sections from the aorta and other arteries of vitamin deficient animals showed no changes in the intima, media, or the adventitia, which might indicate a tendency toward fibrosis or calcification.

The basal metabolism of white rats in relation to the intake of vitamin B. ADDISON GULICK.

The basal metabolism of rats is construed to mean the energy exchange occurring while the animals are prone and apparently asleep during the middle portion of the day, at a chamber temperature in the neighborhood of 30° C. Further comparisons have been made between young male rats eating all they will accept of a diet of starch, casein, butter and salts, and litter mates used as controls, which were fed daily the exact quantity of food eaten day by day by the former rats, but with 0.2 gram of yeast included. Both sets of rats were greatly undernourished by this plan, and gave a severely depressed basal metabolism. The example is given of one rat, starting with a youthful metabolism 153 per cent of the normal adult rate per unit of body surface, becoming 69 per cent of the adult normal after two months without vitamin. At this time the rat also had the paralytic symptoms of beri-beri. One month with yeast included

restored the metabolism to the adult normal rate. The control with vitamin started at 136 per cent of the normal adult rate, dropped during the experimental under-nutrition to 66 per cent of adult normal, and returned to normal in the after-period. Other examples showed a similar result.

Conclusion: The depressed metabolism of a rat receiving deficient vitamin B for long periods is accounted for by the degree of calorie starvation which they develop.

Effect of cations upon blood corpuscles with reference to lysis by hyptonic salt solutions. WINIFRED ASHBY, introduced by L. G. ROWNTREE.

Human blood corpuscles that are incubated for three days in isotonic solutions of potassium or rubidium chlorides at a pH of 7.4 were found upon testing with hypotonic salt solutions to have decreased in resistance, while corpuscles incubated in sodium or lithium salts showed a marked increase in resistance. The effect produced in the presence of any of these cations can be reversed by suitably exchanging the cation, and the reversal is more readily accomplished the farther the effect has proceeded in either direction. In salts of the bivalent cations,—magnesium, calcium, barium and strontium,—a marked increase in resistance occurs. Quantitative analysis shows that changes in resistance, which occur upon incubation in sodium and potassium chlorides, bear a relationship to the potassium content of the corpuscle, the resistance being greater when the potassium content is reduced, and less when it is increased. The increase in resistance which occurs upon incubation in sodium salts does not bear a relationship to sodium content, as this may be either high or low with increased resistance.

It was found that the corpuscles of the horse, guinea pig, and rabbit, which like the human corpuscles have a high potassium content, change in resistance in the presence of the univalent metals in the same way as the human corpuscles, but that the corpuscles of the cat and the dog, which have a high normal sodium content, increase in fragility when incubated in sodium and lithium chlorides and increase in resistance in potassium and rubidium chlorides.

The stimulation of gastric secretion by amino acids and amines. A. C. IVY and A. J. JAVOIS.

We have repeated the experiments of Bickel (Deutsch. med. Wochenschr., 1906, xxxii, 1323) and have confirmed his observations, but offer more conclusive evidence than presented by him, that hydrolyzed meat and casein given by stomach tube stimulate gastric secretion. We have also observed that "gastrin" made by the Koch method stimulates gastric secretion markedly when given by stomach tube in 50 cc. amounts.

The amino acids and amines have been administered chiefly by stomach tube, but in many instances both subcutaneously and intravenously, to Pavlov pouch dogs.

Beta-alanine is a powerful excitant of gastric secretion when given per os in doses of from 0.5 to 1.5 grams. There frequently results as much secretion as is observed after a meal. Intravenously it is negative. Subcutaneously (0.5 to 1.0 gram) it is sometimes slightly positive.

Alpha-alanine (2.0 to 2.5 grams), glycine (1.5 to 2.5 grams), cysteine HCl (1.5 to 2.5 grams), phenylalanine (1.0 to 2.5 grams), are

mild stimulants. Leucine (1.0 to 2.0 grams), tryptophane (600 mgm.), histidine (1 gram), tyrosine (1 gram) and cystine (2 grams) have given doubtful results. Iso-leucine (2 grams) and glutamic acid HCl (1.0 to 2.0 grams) are negative. Lysine picrate (1 gram) is a strong excitant of gastric secretion, but we have shown that the stimulation is due to the picrate portion of the molecule rather than the lysine, as picric acid (free acid neutralized with sodium hydroxide) stimulates gastric secretion when administered by stomach tube or subcutaneously in doses of from 400 to 500 mgm.

Histamine (50 to 150 mgm.), ethylamine HCl (1 to 2.0 grams), and "epinine" (3:4-dihydroxyphenylethylmethylamine) (150 mgm.) given per os are powerful excitants of gastric secretion. "Epinine" (20 to 35 mgm.) subcutaneously stimulates. Ethylamine HCl subcutaneously (500 mgm.) does not stimulate. Tyramine acid phosphate does not stimulate gastric secretion in dogs, it matters not how it is administered. Glucosamine HCl (1 gram) does not stimulate when given per os. Adrenalin (1:3000–1:50,000) in 30 cc. amounts by mouth is rarely positive and subcutaneously it is negative (0.5 to 1.0 cc. of 1:1000).

In all cases the free acidity and some instances the total acidity of the various solutions were neutralized to prevent acid stimulation in the duodenum. (This work has been aided by a grant from the Committee on Scientific Research of the American Medical Association.)

On the presence of iso-agglutinins in the blood of dogs. E. T. McEnery, A. C. Ivy and C. E. Pechous.

Iso-agglutinins are present in the blood of dogs, which confirms the observations of Ottenberg, Kaliski and Friedman (Journ. Med. Research, 1913, xxviii, 141). The titre has varied in our experience from 1:2 to 1:6. When using the serum whole blood glass slide method, agglutination can be seen frequently in from two to three minutes. On standing in the ice-box for seven days the activity of the serum diminishes or disappears. Some serums not causing agglutination may possess a definite tendency to cause rouleaux formation of certain corpuscles.

There have been made 2,270 agglutination tests with 533 positive results. Groups of dogs varying from six to twenty have been used. One group of forty dogs was tested with the hope that the data (1600 tests) would possibly determine the number of blood groups. But it was impossible to form groups. In this group of forty, there were two dogs whose sera did not agglutinate corpuscles and three dogs whose corpuscles were not agglutinable, but there was no dog from which both agglutinins and agglutinogens were absent. Four dogs showed auto-agglutination, which disappeared on warming to 37°C. In four dogs, which were studied for over six months, the agglutinin did not change.

Nine blood transfusions by the "Percy tube" whole blood method have been done, five in which the compatibility was complete (A), three in which the recipient's serum agglutinated the donor's corspucles (B) and one in which the donor's serum agglutinated the corpuscles of the recipient (C). From 200 to 300 cc. of blood were transfused in 10 to 15 kilo dogs, respectively. In group A no symptoms resulted. In group B there were two decided reactions (tremors, hyperpnea, chill, rise or fall in temperature), the third dog showing only a rise of 1.3°F. in rectal temperature. In group C the dog showed hyperpnea and a rise of 1.2°F. in temperature.

The appearance of bilirubin in the urine was followed with positive results in group B.

These results show that in experiments involving blood transfusions and cross-circulation in dogs the compatibility of the bloods should be determined and the only safe procedure is a cross agglutination test.

The resistance of various tissues to gastric digestion. LESTER R. DRAGSTEDT.

A study of the resistance of various living tissues to the digestant action of gastric juice has been made on dogs prepared in the following manner. A large window was made in the anterior wall of the stomach extending nearly to the pylorus. The intestine was then cut across in two places about 6 inches apart and the continuity of the intestine reëstablished by end-to-end anastomosis. The isolated segment opened by an incision the length of the anti-mesenteric border and was carefully stitched into the window of the stomach thus forming a part of the gastric wall. The mucosa of the intestine was exposed to the digestant action of the gastric juice. Great care was taken not to injure the blood supply to the intestinal segment. Flaps of this kind taken from the duodenum, jejunum, ileum and colon were found entirely normal after three to six months' exposure to gastric digestion.

By a somewhat similar technique the spleen and left kidney were implanted into the stomachs of dogs care being taken not to injure the blood supply to the implant. Even after several months' exposure the capsules of these organs were not affected by the gastric digestion. The parenchyma of the spleen and kidney was exposed by first cutting a groove in the surface to be implanted into the stomach. Even when this was done it was found that the splenic pulp and the kidney cortex could resist exposure to the digestant action of normal gastric juice and very little change was evident in these tissues after two and three weeks. Microscopic examination revealed a layer of columnar cells growing out from the stomach to cover the implants.

By experiments in vitro it was found that human and dog gastric juice digested away the hind legs of living frogs. The amount of digestion was in direct proportion to the concentration of free acid in the juice. Artificial frog's gastric juice prepared by extracting the mucosa of the frog's stomach with 0.4 per cent HCl also digested away the hind legs of living frogs of the same species.

Observations on the hemolytic action of ammonium salts. M. H. JACOBS.

Neither of the two usual explanations of the hemolyic action of ammonium salts is entirely satisfactory. That the effect is not due to the free ammonia present in their solutions from hydrolysis is shown by the facts that *a*, the concentration of free ammonia is far too low to have an immediate hemolytic action; *b*, little effect is produced when the solutions are made osmotically suitable by the addition of NaCl or sucrose, and *c*, increasing the amount of free ammonia by increasing the pH of the solution decreases rather than increases the rate of hemolysis. That direct injury of the corpuscles by the ammonium salt is not an important factor is also indicated by the fact that there is an inverse relation between the concentration of the solution and its hemolytic power On the other hand the usual osmotic theory explains neither the ready penetration of NH_4 salts as compared with those of Na and K nor the fact

that hemolysis by NH_4Cl is greatly retarded by slightly increasing the acidity of its solutions. The observed facts may be accounted for by the hypothesis that the corpuscles are relatively impermeable to NH_4 ions as well as to the other cations but are freely permeable to such anions as Cl', OH', etc., and also to NH_4OH or NH_3 molecules. Under these conditions the usual Donnan equilibrium is modified by the slow penetration of undissociated NH_4OH (or NH_3) and its dissociation within the cell with the consequent necessary readjustment in the distribution of Cl' and other ions. As far as the Donnan equilibrium is concerned, increasing acidity favors swelling and increasing alkalinity shrinkage of the corpuscles but in addition there is a secondary effect due to the differential penetration of the NH_4OH or NH_3 molecules which is greater the higher the pH. The actual result produced depends on a combination of both factors. In support of the theory is the fact that at pH's more acid than 4.0, where the amount of free ammonia is negligible, the behavior of NH_4Cl is practically indistinguishable from that of NaCl.

Respiration in Orthoptera. MILTON O. LEE, introduced by R. G. HOSKINS.

Respiration was studied in nine common species of grasshoppers and in cockroaches. It was found that the air does not merely pass in and out of each spiracle as ordinarily taught, but that there is a definite circulation of air through the tracheal system. Air enters through the four anterior pairs of spiracles, traverses the tracheal trunks and is expelled through the last six pairs of abdominal spiracles, and especially through the last, or tenth pair. In quiet respiration practically all the expired gas passes out by the tenth spiracles. Direct observation showed that the valves of the first four pairs of spiracles open during inspiration and close during expiration, while the reverse is true of the valves of the six posterior pairs. When the inspiratory spiracles are closed by cement or immersion in water marked dyspnea followed by complete quiescence was observed. The expired air of the grasshopper was collected and analyzed. The CO_2 content was about 4 per cent. Diffusion of gases through the integument of the insect was found to be too slight to be considered a significant factor in respiration. Water saturated with CO_2 or lactic acid, 1:1000, injected into the body cavity, evoked dyspnea.

A critical study of the nature of the sugar in blood. MAURICE B. VISSCHER.

That the dextrose in the blood may exist in the gamma oxide form, as suggested by Winter and Smith, prompted re-investigation into the problem. Winter and Smith found that if an extract containing the sugar of normal blood were allowed to stand for several days, the optical rotatory power increased, while the copper reduction value remained constant. They found diabetic blood behaved oppositely.

Winter and Smith's procedures were repeated, and their experimental observations were corroborated as far as normal blood is concerned. However, it was found that the same difference which they found existed between normal and diabetic blood could be obtained by varying the H-ion concentration of the filtrate-concentrate of normal blood. If the filtrate is near neutrality, it behaves like their normal blood; if it is quite strongly acid it behaves like their diabetic blood. It was found that the sugar content of the filtrate was not directly proportional to the extent of

change in rotation, indicating that something beside the sugar was responsible for that change.

Winter and Smith's procedures offer no means of eliminating such optically active substances as the amino acids, lactic acid and Beta hydroxybutyric acid. The change noted may easily be due to these substances. The change in rotation observed on standing was not permanent. If the change were actually due to the sugar being converted to a more stable form, why should the effect be so transient?

By using Ur Cl_3 as a precipitating agent results like those Winter and Smith obtained for diabetic blood were obtained with normal blood. Ur Cl_3 gives a very acid filtrate thus explaining the action.

Not enough diabetic patients have been studied to permit a definite statement about the reason for the change in rotatory behavior that Winter and Smith observed. Conflicting results have been obtained in our studies. In one patient, dying in diabetic coma, showing a blood sugar of 0.8 per cent and a Van Slyke of 20 volumes per cent, the blood sugar behaved exactly like normal blood sugar, and it was found that the filtrate was even less acid than that from normal blood, showing that the total fixed base in this patient was increased in spite of a low alkaline reserve.

It is evident that there are many complicating factors which must be eliminated before one is justified in assuming that changes in rotation like those observed are due to the sugar in the filtrate. The observations presented here point rather in the opposite direction.

Effect of caffein, theocin and diuretin on the daily output of urine in rabbits. MAURICE H. REES.

The rabbits used were kept on a uniform intake of 50 cc. of water, by stomach tube, and 20 gram of rolled barley per day. The first series consisted of a four-day control period followed by a four-day test period. During the test period the drugs were given with the water at the beginning of each twenty-four hours. All three drugs produced a marked diuresis on the first day. On the other three days the urinary output was practically normal. With caffein there was some diuresis on the fourth day. Theocin gave the greatest diuresis, three to four times the average amount for the control period. The specific gravity gradually increased but tended to remain below normal throughout the test period.

In the second series the urine was taken hourly by catheterization over a period of ten hours and at the end of twenty-four hours. The normal output of urine ranged from 1 cc. to 5 cc. per hour. Caffein produced a diuresis of 22 cc. during the first hour, 7.5 cc. during the second hour, and a return to normal after eight hours. Theocin produced a diuresis of 61.3 cc. during the first hour, 8 cc. during the second hour, with a return to normal in five hours. Diuretin gave a diuresis of 36 cc. during the first hour, 18 cc. during the second hour, with a return to normal in five hours.

Dosages used: caffein, 20 to 25 mgm. per kilo; theocin, 10 to 16 mgm. per kilo; diuretin, 40 to 45 mgm. per kilo.

A mechanical hypothesis concerning certain anacrotic vibrations in the pulse wave. J. CRIGHTON BRAMWELL, introduced by JOSEPH ERLANGER.

The sphygmogram expresses graphically a pressure-time relation, at a particular point; but the pulse wave may also be considered as a pressure-distance relation, at a particular instant.

In the course of transmission to the periphery, the form of the pulse wave changes, since the different elements of which the wave is composed travel at different rates. Elements which travel quickly tend to catch up others which are travelling more slowly; and, under certain circumstances, portions of the front of the pulse wave may become so steep that their motion is rendered unstable. Such conditions would be likely to give rise to irregular oscillations of pressure in the arteries.

Under the very circumstances where such rapid pressure vibrations might, on purely mechanical grounds, be expected, it is possible to demonstrate their existence in the arteries. It is suggested therefore that certain types of anacrotic vibrations may be produced in a purely mechanical way.

If a series of elements of the front of the pulse wave become unstable simultaneously, the resulting shock in the arteries will be considerable; and this may be one factor in producing the "water-hammer" type of pulse.

Variations in the minute volume of the heart in man during rest and exercise.
MARY E. COLLETT and GÖRAN LILJESTRAND.

These observations were made with the N_2O equilibrium method of Krogh and Lindhard, upon subjects in a semi-recumbent position twelve to fifteen hours after the last meal. Minute volume and oxygen consumption reach the resting level in thirty to forty minutes. The average fasting-resting values for the subject most studied were as follows: minute volume 3.7 L, oxygen consumption 190 cc., ventilation 4.3 L, pulse 64. A light lunch increases the minute volume by 20 to 30 per cent during the first hour: a heavy meal by 40 to 50 per cent. The minute volume three hours after a light meal is still 0.4 L above the fasting level, and 1.0 L above after a heavy meal.

In experiments on the same subject covering three months, a definite periodicity was observed in the fasting-resting values for oxygen consumption and minute volume, with maxima six to ten days before and seven days after the beginning of the menstrual period, and minima on the first and fifteenth days. The difference between the maximal and minimal values were 1 to 2 liters in minute volume and 10 to 30 cc. in oxygen consumption. Ventilation and pulse rate were highest just before the period and lowest on the first day.

Observations were made on three types of exercise, viz., pedalling a stationary bicycle, working the pedals by hand, and climbing a staircase treadmill. It was found that minute volume, pulse-rate and ventilation increase as work increases: but for a given oxygen consumption, the values are higher in some types of work than in others and the difference becomes greater as work increases. At an oxygen consumption of 850 cc. per minute, the minute volume of one subject was 9.5 L for arm work and 6.8 for pedalling; at 1500 cc. oxygen consumption it was 11 L for pedalling and 10.2 L for climbing. An even greater difference was observed for each type of work before and after training. All these differences are apparently correlated with the sense of effort involved. The point of maximum efficiency, as judged by the oxygen used per liter of blood, is reached during fairly heavy work which is well within the capacity of the subject.

The hormone of the ovarian follicle: its action in test animals. EDGAR ALLEN, EDWARD A. DOISY, B. F. FRANCIS, H. V. GIBSON, L. L. ROBERTSON, C. E. COLGATE, W. B. KOUNTZ and C. G. JOHNSTON, introduced by C. W. GREENE.

The subcutaneous injection of liquor folliculi and extracts of follicular contents from ovaries of swine induces typical oestrus in spayed mice and rats (Allen and Doisy, Journ. Amer. Med. Assoc., lxxxi, 819). This definite degree of hyperplasia in the genital tract is equal to the maximum attained in the normal animal under the influence of the ovaries. Three or four 1 cc. injections of an active extract at intervals of four to eight hours return positive results which are apparent forty-eight hours after the first injection. The application of a practicable test which can be followed in the living animal together with the short cycle of our test animals has made these results definite and clear-cut.

Spayed animals in this state of artificially induced oestrus experience mating instincts, sometimes take the initiative in courtship behavior, and accept coitus which is followed as in normal animals by the formation of vaginal plugs. Therefore the follicular hormone is the cause of oestrous growth and secretion and also the ultimate cause of sex instincts in the female. This extract then substitutes for the hormonal function of the ovaries in the absence of pregnancy.

Injections were made to test the effect of the hormone upon immature animals (twenty-four to thirty days old) both normal and spayed. An accelerated growth in the vaginal epithelium resulted in cornification and the opening of the vaginal orifice, not normally patent until the attainment of sexual maturity. Parallel growth changes were present in the uterus. The follicular hormone is therefore responsible for the attainment of puberty involving probably the development of the secondary sexual characters.

Further points of interest include: *1*, Tests of extracts of corpora lutea of both oestrus and pregnancy have proved negative. *2*, Commercial ovarian extracts tested to date have been negative. *3*, Our extracts of liquor dissolved in oil, positive when injected subcutaneously, are negative when administered by stomach tube. *4*, The injection of the hormone into pregnant animals does not cause vaginal cornification and does not interrupt the course of gestation. So far its attempted substitution for ovarian function during pregnancy has failed. *5*, The follicular hormone in much larger doses than required to cause oestrus in the spayed female does not inhibit spermatogenesis in the young male. *6*, Extracts of the contents of cystic follicles are positive.

From *1*, a consideration of the growth of the follicle, *2*, the rapid disappearance of the effects of the hormone soon after ovulation in the normal animal, and *3*, the gel state of the material which distends the newly forming corpus luteum as contrasted with the fluid form of the liquor folliculi, we consider that the follicular hormone is secreted under the influence of the ovum by the follicle cells; that in the ultimate analysis its formation is due to the metabolism of the ovum. Therefore we consider it the principal female sex hormone.

On hyperthermias induced by cerebral lesions and pituitary extract. FRED T. ROGERS.

Complete destruction of the optic thalamus of the pigeon causes diuresis and loss of the ability to regulate body temperature. Intra-peritoneal admininistration of pituitary extract in such a bird causes further diuresis and a rise in body temperature.

Pituitary extract does not cause this temperature effect in normal pigeons either with or without prolonged deprivation of water and food, if ordinary atmospheric conditions are prevalent. If the normal pigeon is deprived of water for two to four days with a dry atmosphere at a temperature of 98° to 100°F. pituitrin will then cause a thermic reaction.

If a normal pigeon is deprived of water for several days and the thalamus is then destroyed, a diuresis follows accompanied by a sharp rise of body temperature which may reach 46°C. followed by the death of the animal. The thermogenic tendency of pituitary extract is counteracted in the normal bird, if tissue dehydration is not present, by the onset of hyperpnea, polypnea, drooping wings and flattening of the feathers against the body, all these being parts of the thermolytic mechanism. These compensating thermolytic reactions do not appear if the thalamus has been destroyed.

The thermic reaction of pituitary extract seems therefore to be due in part to altered distribution of water or to interference with the water exchanges of the tissues. Since pituitary extract causes arteriole and capillary constriction, this interference with the water exchange may be due to lessened blood flow through the tissues and a consequent retention of the heat produced by tissue activity. In part the thermic reaction may depend on increased heat production through stimulation of smooth activity. If these two effects of interference with the water exchange and increased heat production are associated with deficiency of the thalamic temperature regulating centers, the body temperature will rise.

Physiological studies of the marsupial brain. FRED T. ROGERS.

Removal of the major part of the celebral cortex of the opossum in acute experiments, leaving the corpora striata and optic thalamus in situ, is followed acutely by characteristic decerebrate restlessness or continuous walking movements. This restlessness is not inhibited by light, sound or olfactory stimuli. To cutaneous stimuli the animals give avoiding reactions but do not stop their continuous walking movements.

If the major part of the cerebral cortex is removed aseptically, the animal may be kept alive for a period of two weeks. After a few days the continuous restlessness becomes periodic, alternating with periods of rest. Light, sound, olfactory and cutaneous stimuli do not stop this restlessness but food and water at times will do so. Contact of the nostrils with water is followed by drinking. Painful cutaneous stimulation is followed by avoiding reactions. A sudden sharp or a rustling sound causes a sudden start of the entire body or movements of the ears, but they do not inhibit the walking or feeding reactions as in a normal animal. These facts seem to indicate that inhibition of general muscular activities of the body as a whole, in response to extrinsic stimuli, is dependent on a functioning cerebral cortex, and inhibition to internal or visceral stimuli may be carried out by the subcortical centers. Since the opossum brain seems to be one of the most primitive types extant, inhibition seems to be a primitive cortical function.

The odor of fish causes sniffing or smelling movements of the facial muscles but the animal does not seek the food.

The loss of the tempero-parietal cortex of one hemisphere only, causes little or no disturbance. Such an animal seeks food and eats spontaneously, exhibits no paralytic symptoms and gives normal inhibitory responses to external stimuli such as sound and light.

A unilateral lesion of the midbrain, without trauma of the occipital cortex, is followed by forced and continuous muscular movements involving particularly the head, neck and fore-arm muscles of the same side.

Removal of the medio-occipital cortex leads to complete blindness of the contra-lateral eye with no apparent disturbance of vision in the ipse-lateral eye.

The yield of crude insulin by perfusion, percolation and simple extraction. HARRY D. CLOUGH, R. S. ALLEN and JOHN R. MURLIN.

Three distinct methods of treating the pancreas of slaughter-house animals with aqueous solutions for extraction of insulin are described, namely, perfusion, percolation and simple extraction.

For comparison in all three methods pig pancreas was treated with water acidulated with hydrochloric acid and heated to 50°C. In perfusion the strength of acid found most satisfactory is 0.05N, for percolation and simple extraction 0.2N. For simple extraction heating just to 75°C. and immediately cooling has proved more satisfactory than heating to 50°C. for one hour. In all cases the fluid obtained after merely straining is neutralized to a certain reaction varying with the method of extraction. This throws down a heavy precipitate of acid metaproteins.

In agreement with earlier preliminary results on the comparison of the three methods, the yield of crude insulin *in the first filtrate* is greatest after perfusion, next greatest after percolation and least after simple extraction. This depends upon a difference in the kind and amount of protein extracted from the pancreas cells.

By reëxtraction of the first precipitate after perfusion and simple extraction, but not after percolation as here carried out, it is possible to increase the yield of crude insulin enormously. The yield of crude insulin is expressed in Rochester units, i.e., the amount of insulin necessary to lower the blood sugar of a rabbit, which has been fasted 18 hours, is 70 mgm. in two hours. This unit is at least three times as great as the unit adopted by the Insulin Committtee of the University of Toronto. The largest yield of crude insulin was well over 1000 Rochester units, or more than 3000 Toronto units, per kilogram of fresh pancreas.

Attempts to maintain the life of totally pancreatectomized dogs indefinitely by insulin. N. F. FISHER, introduced by A. J. CARLSON.

Some totally pancreatectomized dogs may be kept alive and in fairly good nutrition for at least eight months on insulin. The normal body weight cannot be maintained, and even when the insulin doses are so regulated that little or no sugar appears in the urine, the polyphagia, and some polyuria, is present.

Some totally pancreatectomized dogs on insulin lose weight and die, despite the insulin control of the hyperglycemia and the glycosuria. The determining factor in the temporary or indefinite survival of pancreatectomized dogs on insulin appears to be capacity of the duodenal stump of

the pancreatic duct to regenerate pancreatic tissue, as such regeneration appeared in the two dogs surviving indefinitely, or at least up to eight months, but not in the other dogs.

Insulin probably does not represent the entire pancreas hormone complex, since in the total absence of the pancreas insulin cannot maintain life or does not control all the diabetic symptoms (subnormal weight, polyphagia, polyuria).

The marked degeneration of the liver, and the extreme arterial sclerosis developed in dogs on long insulin management indicate either diabetic processes not controlled by insulin, chronic toxic action of insulin itself, or such toxic action of the other substances in the insulin mixture.

The calorigenic action of adrenalin at rest and at work. WALTER M. BOOTHBY and J. VIDLICKA.

To study the effect of work on the calorigenic action of 0.5 mgm. of adrenalin injected subcutaneously a series of experiments was made on the same individual at rest in bed, walking at the rate of 1200, 3100 and one experiment and one control at 6200 meters per hour. The total calorigenic effect was in bed an average of 19 calories, at the slow walk 22 calories, at the moderate walk 17 calories and in the one experiment at the fast walk 60 calories. Slight and moderate work involving a small proportion of the total mass of muscle does not seem to affect the calorigenic action of adrenalin; we reserve opinion as to the effect of more severe work or work involving a larger proportion of the muscular system until further experiments either confirm or disapprove the apparent increase found at 6200 meters. The subject stated that the injection of the adrenalin did not render the performance of the work easier; in fact about one-half hour after the injection the sensation of fatigue was greater than in experiments without adrenalin. Recovery from the feeling of fatigue at the end of the experiment seemed delayed.

Effect of x-ray and ultra violet radiation on some pharmacodynamic reactions of blood. DAVID I. MACHT and EBEN C. HILL.

Phytopharmacological studies of normal defibrinated blood of the pig were made on seedlings of lupinus albus as described by Macht and collaborators. On comparing the effects of normal blood and the same blood after various radiations distinct changes in the growth of the plants were noted. It was found that heavy radiation of the blood with x-rays rendered it more toxic for plant protoplasm. A few experiments made with blood which was radiated or treated with radium emanations gave very much the same effect. Blood which was radiated with ultraviolet rays by means of a powerful quartz mercury vapor lamp was rendered, if anything, slightly less toxic than the normal controls.

Relation between chemical structure of bile acid and their effects on animal and plant tissues. DAVID I. MACHT, ARTHUR GROLLMAN and O. R. HYNDMAN.

Experiments were made with bile acids and their decomposition products on animals and plants. The zoöpharmacological experiments were directed to an inquiry into the cerebro-spinal effects of the bile acids on white rats as studied in the circular maze. The phytopharmacological experiments were made on the growth of the seedlings of lupinus albus. It was

found that both animal and plant protoplasm were markedly poisoned by glycocholic and taurocholic acid administered in the form of sodium salts. The following products of decomposition were studied: glycocoll, taurine, cholic acid, hyocholic acid and choloidinic acid. It was found, on the one hand, that glycocoll and taurine are but slightly poisonous for both animals and plants, while the various cholic acids were very toxic. The conclusions are that the depressant action of the bile acids for animal and plant protoplasms is to be attributed to the cholic acid component of the molecules.

Vasodilatins are not responsible for the secretagogue action of pancreatic secretin preparations. ARNO B. LUCKHARDT and EDWIN BLONDER.

Dogs under light ether or more or less deep chloretone anesthesia were used. The secretin preparations were extracted from duodenal mucosa with 0.4 per cent HCl acid (1 cc. = 4 grams fresh mucosa). Thirty-two hundredths cubic centimeter per kgm. was injected into the external saphenous vein. Pancreatic juice was collected from the duct of Santorini. Blood pressure records were taken from the carotid. The pylorus was ligated.

We report the following results and conclusions: The secretory and vasodilator actions of secretin preparations are not deteriorated appreciably *1*, by drying duodenal mucosa in air; *2*, by autoclaving mascerated duodenal mucosa prior to extraction by HCl; *3*, by autoclaving active secretin preparations; *d*, by drying active secretin preparations in air on glass plates; *e*, by light; *f*, age, or *g*, action of oxygen allowed to bubble through preparations for three hours.

The vasodilatin action of a given secretin preparation depends on the height of the blood pressure at the time of intravenous injection. If the blood pressure is markedly and permanently lowered (section of spinal cord above the splanchnics, large dose of chloretone, many injections of secretin) a dose of secretin may be without further effect on the blood pressure but produce as copious a flow of pancreatic juice as earlier when the secretagogue action was accompanied by an enormous drop in the general arterial pressure.

Vasodilatins are not responsible for secretion since, *a*, in a given animal the secretory response to different secretin preparations bears no relation to the vadodilatation; *b*, the generalized vasodilatation in the splanchnic area (e.g., after section of the cord above the splanchnics) is not by itself followed by a spontaneous or increased secretion of pancreatic juice; and *c*, preparations of secretin subjected to gastric or pancreatic digestion, or to alkalies (NaOH or Na_2CO_3) lose their secretagogue but not their vasodilatin action.

Do pancreatic secretin preparations represent the normal duodenal hormone? ROY GROGAN and ARNO B. LUCKHARDT.

Dogs under chloretone or barbital anesthesia were used, for the most part, in this work. Secretin preparations were made by extracting the duodenal mucosa with 0.4 per cent HCl (1 cc. = 4 grams fresh mucosa). Injections (0.32 cc. per kgm.) were made through the external saphenous vein. Two tenths per cent to 0.4 per cent HCl was introduced into the duodenum through a duodenostomy.

1. Secretin solutions prepared from different duodena and tested on a given animal showed variations in their efficacy as pancreatic secretagogues.

2. Four tenths per cent HCl introduced into an isolated and denervated loop of bowel (jejunum) induces a powerful secretion of pancreatic juice (confirming Bayliss and Starling).

3. Repeated introduction of 0.2 per cent HCl into the duodenum induced a pancreatic secretion at each introduction. The secretory response became progressively poorer and almost ceased at a time when the intravenous injection of a secretin preparation showed the pancreas still capable of powerful secretory action.

4. The duodenal mucosa, whose secretin content had been reduced to the point of depletion by repeated exposure to 0.2 per cent HCl, contained the usual amount of "secretin" when extracted in the usual way with 0.4 per cent acid and its action compared with secretin preparation from the duodenal mucosa of control animals whose intestine had not been exposed to 0.2 per cent acid.

5. Duodenal mucosa exposed repeatedly *in vivo* to 0.4 per cent HCl yielded on subsequent extraction very little or no "secretin." The gut showed pathological changes as a result of such treatment with the stronger acid.

6. The acid fluid recovered from such loops, on intravenous injection, stimulates the pancreas to marked secretory activity. The secretion occurs in the absence of any general blood pressure changes.

7. The stronger acid extracts the "secretin" *in vivo* without at the same time forming or extracting the vasodilatins.

Nutrition and growth on diets highly deficient or entirely lacking in preformed carbohydrates. THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

White rats were given diets in which the amount of available *preformed* carbohydrate was so small as to be undetectable. The foods contained various proteins and fats in widely different proportions, along with inorganic salts and sources of vitamins A and B. When the energy was furnished essentially in the form of protein rats grew well to 250 grams or more. The food intake was not notably large, indicating that "protein calories" are well used. Diuresis and hypertrophy of the kidneys were often noted. Microscopic examination failed to disclose inflammation or degenerative changes. On diets containing different proteins and varying proportions of fats, with the other dietary essentials but without preformed carbohydrate, good growth was secured in many instances to large size. The absence of preformed carbohydrate did not prevent or alter the prompt recovery of rats which had declined on foods containing no vitamin A, when this factor was supplied. Rats also grew on diets in which the organic foods consisted essentially of protein and fatty acids. The presence of glycerol did not produce any noteworthy advantage. In some of the experiments with foods extremely rich in fats, without apparent detriment to the animals, the ketogenic-antiketogenic ratio greatly exceeded the limit beyond which ketosis is said to arise in man.

Our experiments indicate that in so far as carbohydrate is required for the intermediary metabolism, particularly for the metabolism of fats and the development of energy in muscular contraction, it can be furnished endogenously throughout the period of growth to adult size.

The sugar of the blood. D. A. SCOTT and C. H. BEST, introduced by VELYIEN HENDERSON.

We are concerned in this work with the immediate fate of the sugar which disappears from the blood within a short interval after the subcutaneous or intravenous injection of insulin. Dale (Lancet, May 19, 1923) after a discussion of the available data on this phase of insulin remarks that "sugar disappears but we do not know how." Macleod (Lancet, September 22, 1923) writes "The glucose which disappears is apparently neither oxidized to carbon dioxide and water nor polymerised into glycogen." McCormick, Macleod, Noble and O'Brien report that the heating of blood or of protein-free filtrates if taken from rabbits when the hypoglycemia was most pronounced, caused no greater increase in reducing power than occurs under the same conditions in the normal blood of the same animal. They suggest that the insulin passes out of the blood into the tissues, causes the disappearance of the sugar in the tissues and that consequently sugar is withdrawn from the blood more rapidly than the supply can be replenished.

Our results show that insulin is present in the blood of hypoglycemic rabbits (Proc. Royal Society of Canada, May, 1923) in much greater amounts than in the blood of normal rabbits. We have demonstrated that an extract of 10 cc. of blood from a hypoglycemic rabbit may reduce the blood sugar of a normal rabbit to a very low level. This fact together with the consistent rapid fall in blood sugar during the first half hour after the administration of insulin suggests to us that the mechanism for this rapid disappearance of sugar from the blood is to be found in the blood itself.

We have found that the reducing power of the blood of starved normal rabbits or dogs is usually only very slightly increased by hydrolysis of the blood with dilute sulphuric acid (digest is approximately 1 per cent sulphuric acid) or commercial trypsin. The blood of starved normal rabbits or dogs, which have been rendered hypoglycemic by insulin, when hydrolysed in the same manner as the normal blood shows a pronounced rise in reducing power. The reducing power of this hydrolysed blood is always markedly increased and usually so that it is at least 70 per cent of that of the original normal blood either before or after hydrolysis of the latter.

Our experiments suggest that there is an interesting relationship between the reducing power of hydrolysed blood and the degree of hypoglycemia observed in the blood sample before hydrolysis.

The behavior of glycogen in normal and hyperglycemic animals injected with insulin. N. A. MCCORMICK, E. C. NOBLE and M. K. O'BRIEN, introduced by J. J. R. MACLEOD.

It has been shown that in the depancreatized animal, insulin is able to cause the formation and storage of large amounts of glycogen in the liver. In the normal animal, however, the process is reversed, and the stores of glycogen tend to become depleted after insulin administration. Dudley and Marrian state that with the onset of convulsive symptoms the tissue glycogen has disappeared. In our experience this is not invariably the case. We have investigated the effect of insulin on the liver glycogen after the production in normal rabbits of experimental epinephrin hyperglycemia. Rabbits were subjected to a preliminary period of starvation and then fed for several days on a carbohydrate-rich diet. The quantity of epinephrin

used in each group of observations was kept constant, but the insulin varied according to the behavior of the animal, particularly the blood sugar. The subjoined table reveals that enormous quantities of insulin were sometimes tolerated without convulsions. In all cases the observations were continued during eight hours.

These results show clearly that insulin definitely inhibits the usual glycogenolytic action of epinephrin on the liver.

TABLE 1

NO.	ADRENALIN	ADRENALIN + INSULIN		
	Per cent glycogen	Per cent glycogen	Units insulin used	Blood-sugar in 8 hours
1	1.4	8.12	48	0.18
	1.96	6.40 C	72	0.046
	—	3.64	70	0.050
2	8.56	12.24	110	0.029
	6.60	1.60 C	120	
	1.19 (died)	5.6	140	0.059
3	1.10	3.34	74	0.032
	2.30	10.88 C	130	0.024
	3.10			
4	4.3	9.7	85	
	8.0	1.2 (died)	60	
		10.2	75	0.034
5	2.17	5.6 C	80	0.048 (?)
	1.50	5.0	113	
	0.98	4.7 C	104	0.059

Expers. 1–3 = 2.5 cc. adr. chlor. (1–1000). C = Convulsions. Duration of experiments = 8 hours.
Exper. 4 = 3 cc. adr. chlor. (1–1000).
Exper. 5 = 5.5 cc. adr. chlor. (1–1000).

Respiratory experiments with insulin. B. R. Dickson, G. S. Eadie, J. J. R. Macleod and F. R. Pember.

Respiratory experiments on dogs and rabbits following the injection of insulin show: *a*, in dogs an early rise of the respiratory quotient during the time when the blood sugar is rapidly falling, succeeded by a distinct fall coincident with an enormous increase in ventilation, oxygen consumption, and carbon-dioxide excretion, preceding and culminating in convulsions. At the same time the respiratory and pulse rates increase and the temperature falls. If by giving glucose the blood sugar is prevented from falling below 80 mgm. per 100 cc. we find the early rise in respiratory quotient noted before with a secondary rise sometimes larger, but the increases in ventilation, oxygen consumed and carbon-dioxide excreted, pulse rate, etc., do not occur. *b*. In rabbits there is the early rise in respiratory quotient and the subsequent fall, but the other changes which precede convulsions in dogs do not occur.

The available evidence is against the rise of respiratory quotient's being due to a "blowing-off" of carbon dioxide either from hyperpnea (since it occurs distinctly before the hyperpnea which is accompanied by low quotients), or increased acid production (studies on blood and urine

published elsewhere). There is no direct evidence of the conversion of glucose into a fat-like body.

The later rises in ventilation, etc. are thought to be due to hypoglycemia, probably acting through the central nervous system, exciting increased respiration and muscular tone.

Some physical-chemical aspects of hemolysis. E. C. Mason and P. R. Rockwood.

We are opposed to the belief that hemolysis is dependent upon osmotic pressure changes and to the assumption that the limiting surface of the red blood cell functions as a semipermeable membrane. In support of our view, we offer photographs of cut red blood cells. The untreated, washed cells were sectioned in various planes and photographed ten hours later, under such conditions the hemoglobin-stroma union is still effective.

Vehicles other than stroma may be used to combine with the hemoglobin. We have used kaolin for such a vehicle and have demonstrated that the union depends upon the hydrogen ion concentration. In a series of tubes, with a pH range of 4.4 to 9.2 it was observed that complete union took place within a zone of 6.2 to 6.9 and "hemolysis" occurs on each side of such a zone. The stroma-hemoglobin combination (red blood cells) responds to acid-alkali concentration in a manner analogous to the kaolin-hemoglobin combination. The zone of most complete combination for the stroma-hemoglobin being 7.3 to 8. "Hemolysis" occurs on each side of such a zone.

Evidence is presented to show that it is important to consider the hydrogen ion concentration when doing fragility determinations as the cells may be greatly hemolized on each side of the zone of pH 7.3 to 8.0, while not hemolized within such a zone. In a similar manner one can demonstrate salt protection against acid-alkali hemolysis. A series of tubes, containing washed red blood cells and ranging in pH 4.4 to 10 (made isotonic by sodium chloride) will often show no hemolysis after five hours. However, if diluted at intervals with distilled water hemolysis will be far advanced at each side before present over the neutral zone. Surface tension studies have been conducted on sodium oleate, bile salts and saponin. It was observed that serum protection against hemolysis by sodium oleate parallels the increase of surface tension produced by adding such serum. Hemolysis is a reversible process and the reversal may be accomplished by laking the cells with distilled water and subsequently treating with 5 per cent potassium chloride.

All agents which tend to change the red cell *from* a state of *1*, small mass; *2*, low hydration capacity; *3*, maximum cohesion; and *4*, relative electrical neutrality (all meaning essentially the same) *to* a state of *1*, large mass; *2*, increased hydration capacity; *3*, decreased cohesion (which is accompanied by decreased surface tension); *4*, and away from the isoelectric point, do to the same degree promote hemolysis. Coagulation of blood appears to be just the reverse process of hemolysis.

An ultramicroscopic study of hemolysis. Reed Rockwood and E. C. Mason.

Ultramicroscopic observations of hemolysis by different hemolytic agents together with studies of cell volume seem to indicate that hemolysis, stromatolysis and cell swelling are independent phenomena. The cell

membrane appears to be of minor importance in hemolysis since the cell can be cut into several pieces without the occurrence of hemolysis and since the ultramicroscope shows that the surface of the cell may be destroyed for at least two days before hemolysis occurs. Many of the forms commonly called "crenated" seem to be various stages of stromatolysis. The formation of hemoconia can be observed. Observations with the ultramicroscope confirm the fact, recently demonstrated by Brinkman, that under the proper conditions there may be a true reversal of hemolysis.

The effect of alcohol on the patellar reflex. W. W. TUTTLE, introduced by R. G. HOSKINS.

For the elicitation of the patellar reflex an electrically driven hammer was devised. The apparatus is adjustable so that both the intensity of and intervals between the stimuli can be accurately controlled. The subject sits in a chair provided with adjustments in all directions. A system of pulleys and piano wire from the heel to a stylus records the excursions.

After registering normal kicks for a time, 50 cc. of whiskey in 50 cc. of water were given by mouth. The reflex was found to increase from 17 per cent to more than 2000 per cent in 8 normal subjects. A depressor effect was evident in 2 subjects habituated to alcohol. The augmentor effect persisted from twelve to forty minutes in 6 cases, and more than forty minutes in 2 cases.

The sweat glands studied by the electrical resistance method. CURT P. RICHTER, introduced by D. R. HOOKER.

The resistance offered to the passage of a very small electrical current through the body from the palm of one hand to the palm of the other hand was measured by a string galvanometer. Non-polarizable electrodes made of zinc and a paste mixture of kaolin and saturated zinc sulphate solution were used. Hands were thoroughly cleaned with alcohol and ether. It was found that the resistance offered to the passage of an electrical current from one hand to the other is met in the skin just under the electrodes and does not depend on other parts of the body interposed between the electrodes. This is shown by the fact that the resistance between the palm and the back of the right hand is practically the same as the resistance between the palm of the right hand and the back of the left hand. In the former case the current passes through only the hand, while in the latter case it passes through the hands, arms and the trunk of the body. Systemic hyperdermic injections of adrenalin cause a marked increase in resistance from the palm of one hand to the palm of the other. Pilocarpine greatly decreases the resistance. Atropine increases it. The skin of the palms under the electrodes contains chiefly sweat glands, so that the changes in resistance may be regarded as changes in the condition of the sweat glands. The fact that adrenalin consistently causes an increase in resistance, and pilocarpine a decrease would indicate that the sweat glands are innervated by both sympathetic and parasympathetic nervous systems. This method gives very consistent and accurate results and has been tried out on several hundred individuals in connection with work on the relation between electrical resistance and various emotional conditions.

The relation of the blood sugar to the gastric hunger contractions. E. BULATAO and A. J. CARLSON.

1. Intravenous injection of glucose decreases the gastric hunger contractions. This is not due to osmotic factors, as similar injections of lactose, or hypertonic NaCl do not inhibit the contractions.

2. After insulin injections in normal dogs the gastric hunger contractions and the gastric tonus are augmented to the point of incomplete gastric tetany when the blood sugar falls to 0.085 to 0.075. This gastric tetany is inhibited by intravenous injections of glucose. When the blood sugar reaches the convulsion level (0.045 to 0.035) the gastric tonus and contractions may be periodically inhibited.

3. The augmentation of the gastric tonus and hunger contractions by hypoglycemia may be due to the change in the metabolism of the gastric motor tissues induced by the non-availability of glucose or glycogen in these tissues.